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(54) Title: LACTACYSTIN ANALOGS

(57) Abstract

Compounds related to lactacystin and lactacystin β-lactone, pharmaceutical compositions containing the compounds, and methods of use.

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LACTACYSTIN ANALOGS

BACKGROUND OF THE INVENTION

The invention relates generally to lactacystin and analogues thereof.

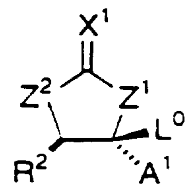
Eukaryotic cells contain multiple proteolytic systems, including lysosomal proteases, calpains, ATP-ubiquitin-proteasome dependent pathway, and an ATP-independent nonlysosomal process. The major neutral proteolytic activity in the cytosol and nucleus is the proteasome, a 20S (700 kDa) particle with multiple peptidase activities. The 20S complex is the proteolytic core of a 26S (1500 kDa) complex that degrades or processes ubiquitin-conjugated proteins. Ubquitination marks a protein for hydrolysis by the 26S proteasome complex. Many abnormal or short-lived normal polypeptides are degraded by the ubiquitin-proteasome-dependent pathway. Abnormal peptides include oxidant-damaged proteins (e.g., those having oxidized disulfide bonds), products of premature translational termination (e.g., those having exposed hydrophobic groups which are recognized by the proteasome), and stress-induced denatured or damaged proteins (where stress is induced by, e.g., changes in pH or temperature, or exposure to metals). In addition, some proteins, such as casein, do not required ubquitination to be hydrolyzed by the proteasome.

The proteasome has chymotryptic, tryptic, and peptidyl-glutamyl peptide hydrolizing activities, i.e., the proteasome can cleave peptides on the carboxyl side of hydrophobic, basic, and acidic residues, respectively.

SUMMARY OF THE INVENTION

The invention relates to novel compounds structurally related to lactacystin and lactacystin β-lactone. The invention also relates to pharmaceutical compositions including lactacystin and lactacystin analogs.

One aspect of the invention is a pharmaceutical composition containing a compound having the formula



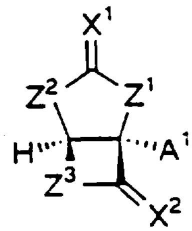
wherein Z^1 is O, S, SO_2, NH, or NR_a, R_a being C_{1-6} alkyl; X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen, X^1 being two singly bonded H when Z^1 is SO_2; Z^2 is O, S, NH, NR_d, CHR^1, or $CHOR^1$ in the *(R)* or *(S)* configuration, wherein R_d is C_{1-6} alkyl and R^1 is H, halogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, NR_dR_e (except where Z^2 is $CHOR^1$), or the side chain of any naturally occurring α-amino acid, or R^1 and R^2 taken together are a bivalent moiety, provided that when R^1 and R^2 are taken together, Z^1 is NH or NR_a and Z^2 is CHR^1; R_e being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, and the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl, where the H in CHR^1 is deleted when R_1 and R_2 taken together form a C_{3-8} heteroaryl or C_{6-12} aryl; R^2 is C_{1-6}

alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, azido, C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H, C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1 and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl, where the H in CHR^1 is deleted when R_1 and R_2 taken together form a C_{3-8} heteroaryl or C_{6-12} aryl; A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and L^O is an organic moiety having 1 to 25 carbon atoms, 0 to 10 heteroatoms, and 0 to 6 halogen atoms; and a pharmaceutically acceptable carrier.

A second aspect is a pharmaceutical composition comprising a compound having the following formula



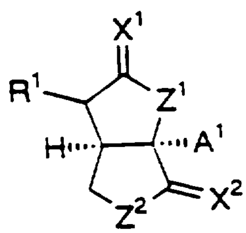
wherein Z^1 is O, S, SO_2, NH, or NR_a, R_a being C_{1-6} alkyl; X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen, provided that when Z^1 is SO_2, X^1 is two singly bonded H; Z^2 is CHR^1 in the *(R)* or *(S)* configuration, R^1 being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl); Z^3 is O, S, NH, or NR_j, wherein R_j is C_{1-6} alkyl; X^2 is O or S; and A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

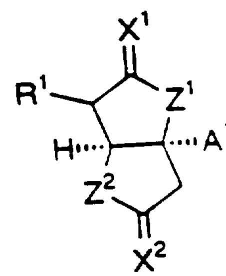
wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C{=}NOR_h$, $-C{=}NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14}

arylalkylene, or deleted; and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and a pharmaceutically acceptable carrier.

A third aspect is a pharmaceutical composition comprising a compound having one of the following formulae



or



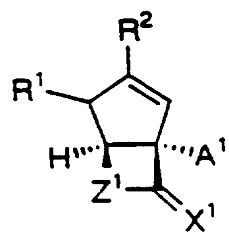
wherein Z^1 is NH or NR_a, R_a being C_{1-6} alkyl; X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen; Z^2 is O, S, NH, or NR_j, wherein R_j is C_{1-6} alkyl; R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occurring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen); X^2 is O or S; and A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, -$C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)*

or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and a pharmaceutically acceptable carrier.

A fourth aspect is a pharmaceutical composition containing a compound having the following formula



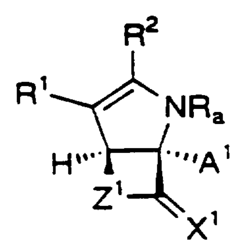
wherein Z^1 is O, S, NH or NR_j, R_j being C_{1-6} alkyl; X^1 is O or S; R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl); R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-12} aryl, C_{3-8} heteroaryl, or halogen; X^2 is O or S; and A^1 is H, the

side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)-oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and a pharmaceutically acceptable carrier.

A fifth aspect is a pharmaceutical composition comprising a compound having the following formula



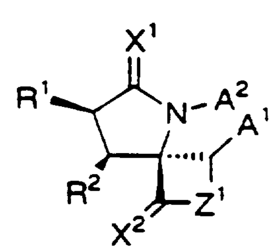
wherein Z^1 is O, S, NH or NR_j, R_j being C_{1-6} alkyl; X^1 is O or S; R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of

a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} hetero-cyclic radical, or halogen); R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-12} aryl, C_{3-8} heteroaryl, or halogen; R_a is C_{1-6} alkyl; and A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)-oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and a pharmaceutically acceptable carrier.

A sixth aspect is a pharmaceutical composition containing a compound having the formula



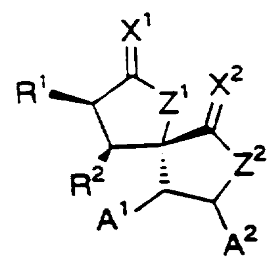
wherein X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen; Z^1 is O, S, NH, or NR_a, R_a being C_{1-6} alkyl; R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of any naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} halo-alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen); or R^1 and R^2 taken together are a bivalent moiety which forms a C_{3-8} cyclo-alkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl; R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, azido, C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H, C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1 and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl; X^2 is O or S; and A^1 is in the *(R)* or *(S)* configuration, and each of A^1 and A^2 is independently H, the side chain of any naturally occurring amino α-acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C{=}NOR_h$, $-C{=}NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is

straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)-oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and a pharmaceutically acceptable carrier.

A seventh aspect is a pharmaceutical composition containing a compound having the following formula



wherein Z^1 is NH or NR_a, NR_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen;

Z^2 is O, S, NH, or NR_j, R_j being C_{1-6} alkyl;

R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of any naturally occuring amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen); or R^1 and R^2 taken together are a bivalent moiety which forms a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl; R^2 is H, C_{1-6} alkyl, C_{1-6}

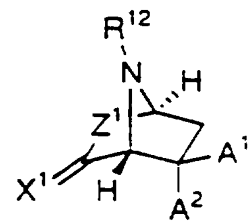
haloalkyl, C_{2-6} alkenyl, azido, C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H, C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1 and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl; X^2 is O or S; and each of A^1 and A^2 is independently in the *(R)* or *(S)* configuration, and is independently H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C{=}NOR_h$, $-C{=}NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)-oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and a pharmaceutically acceptable carrier.

An eighth aspect is a pharmaceutical composition

containing a compound of the formula



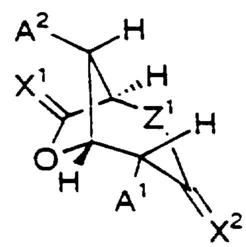
wherein Z^1 is O, NH, or NR_a, NR_a being C_{1-6} alkyl; X^1 is O, S, CH_2, or two singly bonded H; each of A^1 and A^2 is independently H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)-oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and R^{12} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl; and a pharmaceutically acceptable carrier.

A ninth aspect is a pharmaceutical composition

comprising a compound having the formula



wherein Z^1 is NH, or NR_a, NR_a being C_{1-6} alkyl; each of X^1 and X^2, independently, is O or S; each of A^1 and A^2 is independently in the *(R)* or *(S)* configuration, and is independently H, the side chain of any naturally occurring amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)-oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and a pharmaceutically acceptable carrier.

Many of the compounds described above are novel compounds; the novel compounds are also claimed. The invention also encompasses lactacystin analogues that can be made by the synthetic routes described herein, and

methods of treating a subject having a condition mediated by proteins processed by the proteasome by administering (e.g., orally) to a subject an effective amount of a pharmaceutical composition disclosed herein. These methods include treatments for cancer, inflammation (e.g., inflammatory responses associated with allergies, bone marrow or solid organ transplantation, or disease states), psoriasis, and restenosis.

The compounds disclosed herein are highly selective for the proteasome, and do not inhibit other proteases such as trypsin, α-chymotrypsin, calpain I, calpain II, papain, and cathepsin B.

Other features or advantages of the present invention will be apparent from the following detailed description, and also from the appending claims.

DETAILED DESCRIPTION OF THE INVENTION

Terms

The term "naturally occurring amino acid" is meant to include the 20 common α-amino acids (Gly, Ala, Val, Leu, Ile, Ser, Thr, Asp, Asn, Lys, Glu, Gln, Arg, His, Phe, Cys, Trp, Tyr, Met and Pro), and other amino acids that are natural products, such as norleucine, ethylglycine, ornithine, methylbutenylmethylthreonine, and phenylglycine. Examples of amino acid side chains include H (glycine), methyl (alanine), $-(CH_2-(C=O)-NH_2$ (asparagine), $-CH_2-SH$ (cysteine), and $-CH(OH)CH_3$ (threonine).

The term "inhibitor" is meant to describe a compound that blocks or reduces the activity of an enzyme (e.g. the proteasome, or the X/MB1 subunit or α-chain of the 20S proteasome). An inhibitor may act with competitive, uncompetitive, or noncompetitive inhibition. An inhibitor may bind reversibly or irreversibly, and therefore the term includes compounds which are suicide

substrates of an enzyme. An inhibitor may modify one or more sites on or near the active site of the enzyme, or it may cause a conformational change elsewhere on the enzyme. Thus, some compounds disclosed herein (e.g., where L^0 is an oxiranyl or aldehyde group) react with the enzyme by bonding to the carbon atom corresponding to C4 of lactacystin (e.g., resulting in a C4 having a hydroxyl or thiol), while other compounds react with the enzyme to release a leaving group (e.g., L^1), corresponding to an acylation.

An alkyl group is a branched or unbranched hydrocarbon that may be substituted or unsubstituted. Examples of branched alkyl groups include isopropyl, sec-butyl, isobutyl, tert-butyl, sec-pentyl, isopentyl, tert-pentyl, isohexyl. Substituted alkyl groups may have one, two, three or more substituents, which may be the same or different, each replacing a hydrogen atom. Substituents are halogen (e.g., F, Cl, Br, and I), hydroxyl, protected hydroxyl, amino, protected amino, carboxy, protected carboxy, cyano, methylsulfonylamino, alkoxy, acyloxy, nitro, and lower haloalkyl. Similarly, cycloalkyl, aryl, arylalkyl, alkylaryl, heteroaryl, and heterocyclic radical groups may be substituted with one or more of the above substituting groups. Examples of cycloalkyl groups are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. An aryl group is a C_{6-20} aromatic ring, wherein the ring is made of carbon atoms (e.g., C_{6-14}, C_{6-10} aryl groups, wherein C_8 aryl may be an alkylaryl such as 2,3-dimethylphenyl or an arylalkyl such as phenylethyl, or an alkylarylalkyl such as o-methyl-benzyl). Examples of haloalkyl include fluoromethyl, dichloromethyl, trifluoromethyl, 1,1-difluoroethyl, and 2,2-dibromoethyl.

A heterocyclic radical contains at least one ring structure which contains carbon atoms and at least one

heteroatom (e.g., N, O, S, or P). A heteroaryl is an aromatic heterocyclic radical. Examples of heterocyclic radicals and heteroaryl groups include: thiazolyl, thienyl, furyl, 1-isobenzofuranyl, 2H-chromen-3-yl, 2H-pyrrolyl, *N*-pyrrolyl, imidazolyl, pyrazolyl, isothiazolyl, isooxazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyradazinyl, indolizinyl, isoindolyl, indolyl, indazolyl, purinyl, phthalazinyl, cinnolinyl, and pteridinyl. A heterocylic radical may be attached to another moiety via a carbon atom or a heteroatom of the heterocyclic radical.

A (C_n alkyl)oxycarbonyl group has the formula R-O-(C=O)-. (C_{1-6} alkyl)oxycarbonyl, therefore, includes methoxycarbonyl and hexyloxycarbonyl. A C_{1-10} acyl group as used herein is of the formula -(C=O)-L_3 and contains 1 to 10 carbon atoms (e.g., C_{1-6}) and 1-5 heteroatoms (at least one oxygen). Examples of such acyl groups include formyl, acetyl, benzyloxycarbonyl, *tert*-butoxycarbonyl, trifluoroethyloxycarbonyl, thiobenzoyl, phenylamidocarbonyl, and 4-nitrophenoxycarbonyl.

An alkylene is a bivalent radical derived from alkanes by removing two hydrogens from two different carbon atoms. Examples of alkylenes include -CH_2-CH(R)-CH_2 and 1,4-cyclohexylene. An alkylidene is a bivalent radical derived from alkenes by removing two hydrogens from the same carbon atom, such as 1-propanyl-3-ylidene (=CH-CH_2-CH_2-).

An aromatic carbon atom, as used herein, is a carbon atom within an aromatic system such as benzene or naphthalene or a heteroaromatic system such as quinoline. Examples of nonaromatic carbon atoms include the carbons atoms in R-(C=O)-R, -CH_2Cl, -CH_2- and R-(C=O)-O-R. A fragment formula weight is the combined atomic weight of the fragment or moiety indicated. For example, the

fragment formula weight of methyl is 15 and the fragment formula weight of hydroxyl is 17.

A leaving group departs from a substrate with the pair of electrons of the covalent bond between the leaving group and the substrate; preferred leaving groups stabilize those electrons via the presence of electron withdrawing groups, aromaticity, resonance structures, or a combination thereof. Examples include halide (I and Br are preferred), mesylate, trifluoromethanesulfonate, *p*-toluenesulfonate, p24-nitrobenzensulfonate, benzoate, *p*-nitrobenzoate, *p*-nitrobenzyl, and C_{2-5} haloalkylcarbonyloxy such as trifluoroacetate.

Numerous thiol-, amino-, hydroxy- and carboxy-protecting groups are well-known to those in the art. In general, the species of protecting group is not critical, provided that it is stable to the conditions of any subsequent reaction(s) on other positions of the compound and can be removed at the appropriate point without adversely affecting the remainder of the molecule. In addition, a protecting group may be substituted for another after substantive synthetic transformations are complete. Clearly, where a compound differs from a compound disclosed herein only in that one or more protecting groups of the disclosed compound has been substituted with a different protecting group, that compound is within the invention. For example, where a compound differs from a compound disclosed herein only in that one or more hydrogens from a thiol-, amino-, or hydroxyl- moiety (or a hydrogen or hydroxyl from a carboxyl moiety) has been substituted with a protecting group to form a carboxyl or sulfonyl ester, that protected compound is within the invention. Sulfonyl esters include alkylsulfonyls (e.g., methylsulfonyl or mesyl) and arylsulfonyls (e.g., tosyl). Further examples and conditions are found in T.W. Greene, *Protective*

Groups in Organic Chemistry, (1st ed., 1981; 2nd ed., 1991, T.W. Greene and P.G.M. Wuts).

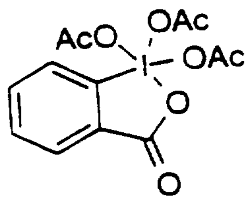
The invention also encompasses isotopically-labelled counterparts of compounds disclosed herein. An isotopically-labelled compound of the invention has one or more atoms replaced with an isotope having a detectable particle- or x-ray-emitting (radioactive) nucleus or a magnetogyric nucleus. Examples of such nuclei include ^{2}H, ^{3}H, ^{13}C, ^{15}N, ^{19}F, ^{29}Si, ^{31}P, ^{32}P and ^{125}I. Isotopically-labelled compounds of the invention are particularly useful as probes or research tools for spectrometric analyses, radioimmunoassays, binding assays based on γ- or β-scintillation, fluorography, autoradiography, and kinetic studies such as inhibition studies or determination of primary and secondary isotope effects.

The following abbreviations are used in the synthetic description:

AIBN, 2,2'-azobis(isobutyronitrile); Bn, benzyl; BOP-Cl, bis(2-oxo-3-oxazolidinyl)phosphinic chloride; Bu_2BOTf, dibutylboron triflate; CDI, *N,N'*-carbonyl-diimidazole; Cp, cyclopentadienyl; DBU, 1,8-diazabicyclo-[5.4.0]undec-7-ene; DCC dicyclohexylcarbodiimide; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DEAD, diethylazodicarboxylate; DIBAL-H diisobutylaluminum hydride; DMF, dimethylformamide; Gilbert reagent, dimethyl diazomethylphosphonate; LDA, lithium diisopropylamide; LiHMDS, lithium hexamethyldisilazamide; mesylate, methanesulfonic acid ester; Mitsunobu reagents, (DEAD, PPh_3, and nucleophile); NMO, *N*-methylmorpholine-*N*-oxide; Ph, phenyl; PhFl, 9-phenyl-9-fluorenyl; Ph_2NTf, *N*-phenyltrifluoromethanesulfonimide; Swern oxidation reagents ($(COCl)_2$, DMSO, Et_3N); TBAF, tetrabutylammonium fluoride; TBS, *tert*-butyldimethylsilyl; TCDI, thiono-*N,N'*-carbonyldiimidazole; Tf_2O, trifluoromethane-

sulfonic acid anhydride; TMS, trimethylsilyl; triflate, triflurومethane sulfonate ester; and TsCl, *p*-toluene-sulfonyl chloride.

The following reagents are also used:



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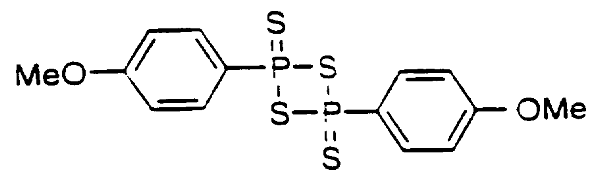
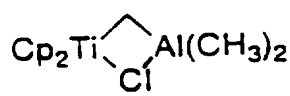
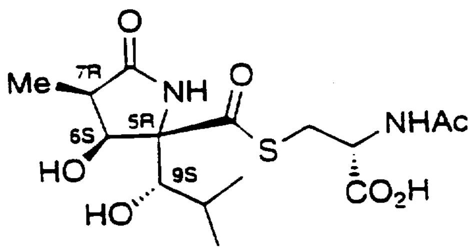


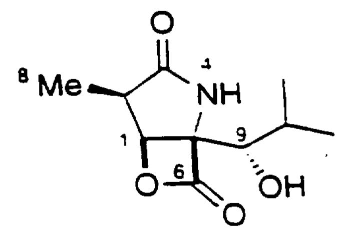
Lawesson's reagent



Tebbe reagent

The invention is based, in part, on the structure-function information disclosed herein which suggests the following preferred stereochemical relationships. Note that a preferred compound may have one, two, three, or more stereocenters having the indicated up-down (or β-α where β as drawn herein is above the plane of the page) or (*R*)-(*S*) relationship. In some embodiments, preferred configurations are 7(*R*), 9(*S*), 6(*S*), or combinations thereof. In lactacystin analogs, R^2 and L^0 preferably have a *syn* relationship to allow formation of a lactone or lactam-type intermediate, which is believed to be related to the activity of the compounds disclosed herein as proteasome inhibitors. However, it is not required that every stereocenter conform to the structures below.





Lactacystin Lactacystin β-lactone

A person of skill will recognize that the compounds described herein preserve certain stereochemical and electronic characteristics found in either lactacystin or lactacystin β-lactone. For example, the hydroxyisobutyl group and the configuration of the hydroxyl group on C9 are believed to be important for recognition of the target, as are the configurations of the C6 hydroxyl and the C7 methyl of the γ-lactam ring. However, the N-acetylcysteine moiety is not required for activity. Moieties such as R^2, R^1, and particularly A^1 (e.g., where A^1 is a side chain of a naturally occurring α-amino acid such as Val, Leu, Lys, and Phe) can be modified to control selectivity for the proteasome, and selectivity for a particular peptidase activity of the proteasome. In combination, these moieties simulate certain peptides or proteins processed or degraded by the proteasome (i.e., are peptidomimetics).

The invention is also based, in part, on the finding that lactacystin and lactacystin β-lactone are highly selective for the X/MB1 subunit and α-chain of the proteasome and do not inhibit the activity of proteases such as trypsin, α-chymotrypsin, calpain I, calpain II,

cathepsin, and papain. Such selectivity is useful to formulate a pharmaceutical composition with fewer side effects and to evaluate basic research results involving any subunit of the proteasome in conjunction with the selective inhibition of the X/MB1 subunit and α-chain.

Turning to the novel compounds described by the formulas of compounds contained within the pharmaceutical compositions, several embodiments are next considered.

Embodiments of the first aspect include compounds having one or more of the following limitations. In one limitation L^1 is linked by an oxygen or preferably sulfur atom to the carbon atom bonded to X^2. Such an L^1 forms a thioester, thus providing a good leaving group. The precise nature of the thioester group is generally not critical. In initial experiments, the magnitude of activity measured by $K_{obs}/[I]$ for both large and small thioesters was about the same, whether the thioester terminus was a simple arylalkyl group or a more complex, branched moiety with intervening amide linkages and terminal carboxylic acid, esters, or aminocarbonyl groups. In one embodiment, Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 or $CHOR^1$, R^1 being H, halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl; R^2 is OR_f; and L^0 is $-(C{=}X^2)-L^1$, X^2 being O and L^1 being linked by a sulfur atom to the carbon atom bonded to X^2. In another embodiment, L^1 is selected from substituted or unsubstituted C_{6-20} arylalkylcarbonylthio, C_{6-20} arylthio, C_{2-8} alkylcarbonylthio, and C_{1-12} alkylthio. As defined above in the "Terms" section, arylthio includes arylthio, alkylarylthio, arylalkylthio, and alkylarylalkylthio groups.

In other limitations, A^1 is C_{5-20} alkyl when L^0 is carboxyl or (C_{1-4} alkyl)oxycarbonyl and A^1 is alkyl; only one of A^1 and L^0 is selected from carboxyl and (C_{1-4} alkyl)oxycarbonyl; A^1 cannot be H; L^1 has at least 3 carbon atoms when Z^1 and Z^2 are both NH, and A^1 is

methoxy; when R^1 and R^2 are taken together, Z^2 is CR^1; and when one of A^1 and L^O is (C_{1-4} alkyl)oxycarbonyl or carboxyl, the other of A^1 and L^O has a fragment formula weight of at least 20. Novel compounds of the first, fifth, and seventh aspects generally do not have A^1 being H.

Further embodiments of the first aspect, when Z^1 is NH and Z^2 is (*R*) CHR^1, are compounds wherein: L^1 has between 0 and 3 nonaromatic acyclic carbon atoms when there are 5 heteroatoms; L^1 has between 6 and 15 nonaromatic acyclic carbon atoms when there is only one oxygen atom; and L^1 has 0, 1, 3, or 5 nonaromatic carbon atoms when there are three halogen atoms.

Further embodiments of the first aspect include a compound wherein Z^2 is CHR^1 and Z^1 is NH or NR_a; wherein L^O is C_{2-16} oxiranyl; L^1 is hydroxy, C_{1-12} alkoxy, C_{1-12} alkyl-sulfonyloxy, C_{6-20} arylsulfonyloxy, C_{7-20} arylalkyl, C_{6-20} aryloxy, C_{6-20} arylalkylcarbonyloxy, C_{2-8} alkyl-carbonyloxy, C_{2-8} alkylcarbonylthio, C_{1-12} alkylthio, C_{6-20} arylalkylcarbonylthio, or C_{6-20} arylthio; or L^2 is H, C_{1-2} haloalkyl, or C_{1-6} alkyl. In addition, Z^2 is O, S, NH, or NR_d. In another embodiment, Z^2 is O, S, NHJ, NR_d, or CHR^1; and R_h is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl.

Further embodiments of the first aspect include compounds wherein Z^1 is O, S, or SO_2; wherein Z^2 is O, S, NH, or NR_d; wherein X^1 is CH_2, CHR_b, or $C(R_b)(R_c)$; wherein R^2 is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or halogen; wherein R^2 is OR_f, SR_f, or NR_fR_g; wherein Z^2 is in the beta (above plane of page) configuration; wherein CHX^4 is in the alpha (below plane of paper) configuration; wherein R^2 is in the beta configuration; or combinations thereof.

The invention also provides a pharmaceutical composition wherein L^O is H or $-(C=X^2)-L^1$, X^2 being O and

L^1 being linked by an oxygen or sulfur atom to the carbon atom bonded to X^2; where only one of A^1 and L^0 is selected from carboxyl and (C_{1-4} alkyl)oxycarbonyl; wherein L^1 is hydroxy, C_{1-12} alkoxy, C_{1-12} alkylsulfonyloxy, C_{6-20} arylsulfonyloxy, C_{7-20} arylalkyl, C_{6-20} aryloxy, C_{6-20} arylalkylcarbonyloxy, C_{2-8} alkylcarbonyloxy, C_{6-20} arylalkylcarbonylthio, C_{6-20} arylthio, C_{2-8} alkyl-carbonylthio, or C_{1-12} alkylthio.

The invention also provides embodiments wherein Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 or $CHOR^1$, R^1 being H, halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl; R^2 is OR_f; and L^0 is $-(C{=}X^2)-L^1$, X^2 being O and L^1 being linked by a sulfur atom to the carbon atom bonded to X^2. Individual compounds include lactacystin, clasto-lactacystin trifluoroethyl ester, descarboxylactacystin, lactacystin amide, and phenylacetyl lactacystin.

Embodiments of the second aspect include compounds wherein CHX^4 is in the *(S)* configuration when X^4 is hydroxyl and $-(CH_2)_n-R^3X^3$ is isopropyl; wherein the moiety $-(CH_2)_n-R^3X^3$ has between 5 and 20 carbon atoms when X^4 is hydroxyl, m is 0, and Z^1 is NH; wherein Z^1 is NH or NR_a; wherein Z^1 is O, S, or SO_2; wherein Z^1 is NR_a; wherein X^1 is CH_2, $CH(R_b)$, or $C(R_b)(R_c)$; wherein Z^2 is in the beta (above plane of page) configuration; wherein CHX^4 is in the alpha (below plane of paper) configuration; wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, or halogen; wherein R^1 is a side chain of a naturally occurring α-amino acid, OR_d, SR_d, or NR_dR_e; or combinations of the above.

Another embodiment of the second aspect includes compounds wherein Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 in the *(R)* or *(S)* configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occurring amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e,

independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl); Z^3 is O or NH; X^2 is O or S; and A^1 is the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-Y-(CH_2)_n-R^3X^3$$

wherein Y is C=O or CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, or OR_h, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, or C_{6-10} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

The invention also provides a compound wherein Z^1 is NH and Z^3 is O; or a compound wherein each of Z^1 and Z^3 is NH; or a compound wherein at least four (e.g., at least 5, or all) of the following limitations apply: Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 in the (R) or (S) configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, hydroxyl, or OR_d, R_d being C_{1-6} alkyl; Z^3 is O or NH; X^2 is O; Z^3 is O or NH; and A^1 is the side chain of any naturally occurring α-amino acid, or CHX^4, X^4 being halogen, methyl, halomethyl, or OR_h, R_h being selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; m is 0, 1, or 2 and n is 0 or 1; and X^3 is H.

The invention also provides a pharmaceutical composition wherein Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 in the *(R)* or *(S)* configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring

α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl); Z^3 is O or NH; X^2 is O or S; and A^1 is the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-Y-(CH_2)_n-R^3X^3$$

wherein Y is C=O or CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, or OR_h, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, or C_{6-10} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

Another embodiment includes a compound wherein X^2 is O, and A^1 is the side chain of any naturally occurring α-amino acid, or X^4 is halogen, methyl, halomethyl, or OR_h, R_h being selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; m is 0, 1, or 2 and n is 0 or 1; and X^3 is H. Individual lactone analogs include clasto-lactacystin β-lactone.

Embodiments of the third aspect include compounds wherein: when X^1 is 2 singly bonded H and R^1 has only one oxygen, then the fragment formula weight of R^1 is at least 30 atomic mass units; when X^1 is 2 singly bonded H and R^1 is 2 H, and A^1 is alkyl, then A^1 has at least 3 carbon atoms; A^1, R^1 and X^1 taken together have at least one carbon atom, halogen, or heteratom; when X^1 is two singly bonded H, and R^1 is H, X^2 is O, and R_h is alkyl, then R_h is C_{4-6} alkyl; and when n is 2, R_h is C_{1-6} haloalkyl.

Further embodiments of the third aspect include compounds wherein: X^1 is CH_2, two singly bonded H, $CH(R_b)$ or $C(R_b)(R_c)$; Z^2 is O or S; Z^2 is NH or NR_j; R^1 is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, or halogen; R^1 is a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e; A^1 cannot be H; R^1 is in the beta configuration; X^4 is in the alpha configuration; or combinations thereof.

Embodiments of the fourth aspect include compounds wherein: Z^2 is O or S; Z^2 is NH or NR_j; X^1 is O or S; R^1 is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, or halogen; R^1 is a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or $NR_dR_{e;}$; R^2 is H, C_{1-3} alkyl, C_{1-3} haloalkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, C_{6-12} aryl, C_{3-8} heteroaryl, or halogen; R^2 is H, C_{3-6} alkyl, C_{3-6} haloalkyl, C_{4-6} alkenyl, C_{4-6} alkynyl, C_{6-12} aryl, or C_{3-8} heteroaryl; A^1 cannot be H; R^1 is in the beta configuration; X^4 is in the alpha configuration; or combinations of the above.

Further embodiments of the fifth aspect are compounds wherein: Z^1 is O or S; Z^2 is NH or NR_j; R^1 is H, C_{1-3} alkyl, C_{1-3} haloalkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, or a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e; R^1 is H, C_{4-6} alkyl, C_{4-6} haloalkyl, C_{4-6} alkenyl, C_{4-6} alkynyl, halogen, or a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e; R^2 is H, C_{1-3} alkyl, C_{1-3} halo-alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, C_{6-12} aryl, C_{3-8} heteroaryl, or halogen; R^2 is H, C_{4-6} alkyl, C_{4-6} haloalkyl, C_{4-6} alkenyl, C_{4-6} alkynyl, C_{6-12} aryl, or C_{3-8} heteroaryl;n R_a is C_{1-3} alkyl; A^1 cannot be H; X^4 is in the alpha configuration; or combinations thereof.

Embodiments of the sixth aspect are compounds wherein: X^1 is O or S; X^1 is CH_2, two singly bonded H, $CH(R_b)$, or $C(R_b)(R_c)$; Z^1 is O or S; Z^1 is NH or NR_a; R^1 is

C_{1-3} alkyl, C_{1-3} haloalkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, halogen, or a side chain of any naturally occuring α-amino acid; R^1 is OR_d, SR_d, or NR_dR_e; R^1 is C_{4-6} alkyl, C_{4-6} haloalkyl, C_{4-6} alkenyl, C_{4-6} alkynyl, a side chain of any naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e; R^1 and R^2 taken together are a bivalent moiety; R^1 is in the beta configuration; R^2 is in the beta configuration; R^2 is C_{1-3} alkyl, C_{1-3} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, or halogen; R^2 is C_{4-6} alkyl, C_{4-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, or C_{3-8} heterocyclic radical; A^1 has a higher fragment formula weight than A^2; A^1 has a lower fragment formula weight than A^2; A^1 is in the (R) configuration; A^1 is in the (S) configuration; only one of A^1 and A^2 is H; A^1 is a side chain of any naturally occuring α-amino acid; or combinations thereof.

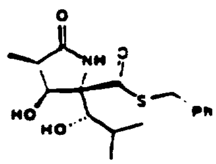
Embodiments of the seventh aspect are compounds wherein: X^1 is O or S; X^1 is CH_2, two singly bonded H, $CH(R_b)$ or $C(R_b)(R_c)$; Z^2 is O or S; Z^2 is NH or NR_j; R^1 is C_{1-3} alkyl, C_{1-3} haloalkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, hydroxyl, halogen, or a side chain of any naturally occuring α- amino acid; R^1 is OR_d, SR_d, or NR_dR_e; R^1 is C_{4-6} alkyl, C_{4-6} haloalkyl, C_{4-6} alkenyl, C_{4-6} alkynyl, a side chain of any naturally occuring α-amino acid; R^1 and R^2 taken together are a bivalent moiety; R^2 is C_{1-3} alkyl, C_{1-3} haloalkyl, C_{2-3} alkenyl, azido, C_{2-3} alkynyl, hydroxyl, or halogen; R^2 is OR_f, SR_f, $NR_fR_fR_g$, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(RS_f)$; R^2 is C_{4-6} alkyl, C_{4-6} haloalkyl, C_{4-6} alkenyl, azido, C_{4-6} alkynyl, OR_f, SR_f, $NR_fR_fR_g$, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(RS_f)$; R^1 and R^2 taken together are a bivalent moiety; R^1 is in the alpha configuration; R^1 is in the beta configuration; R^2 is in the alpha configuration; R^2 is in the beta configuration; A^1 is in the alpha configuration; A^2 is in the beta configuration; or combinations thereof.

One embodiment of the eighth aspect is a compound where A^1, A^2, R^{12}, and X^1 taken together have at least one carbon atom and one heteratom. Further embodiments of the eighth aspect are compounds wherein: Z^1 is NH, or NR_a; X^1 is O or S; X^1 is CH_2, or two singly bonded H; R^{12} is H, C_{1-3} alkyl, C_{1-3} haloalkyl, C_{2-3} alkenyl, or C_{2-3} alkynyl; R^{12} is C_{4-6} alkyl, C_{4-6} haloalkyl, C_{4-6} alkenyl, or C_{4-6} alkynyl; where A^1, A^2, R^{12}, and X^1 taken together have at least one carbon atom and one heteratom; where the fragment formula weight of A^1 is greater than the fragment formula weight of A^2 (e.g., by at least 30 or 60); where the fragment formula weight of A^2 is greater than the fragment formula weight of A^1 (e.g., by at least 15 or 50); or combinations thereof.

Embodiments of the ninth aspect are compounds wherein A^1 is a side chain of any naturally occurring α-amino acid; wherein A^1 has a fragment formula weight of at least 50 (e.g., 70, 100, or 120).

Examples of individual compounds described herein are provided in Tables A, B, and C.

Table A



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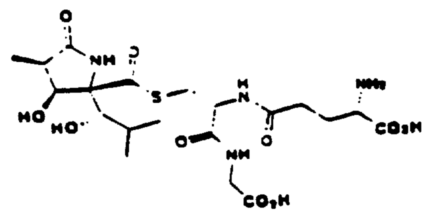

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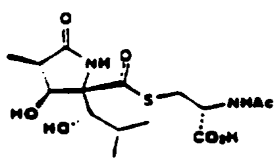

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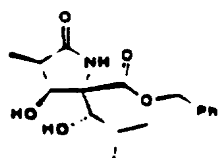

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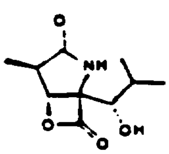

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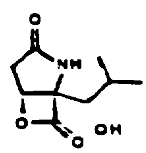

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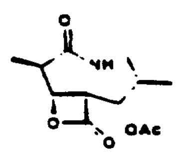

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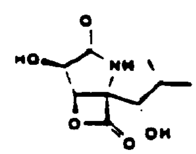

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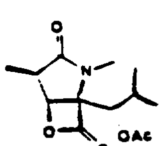

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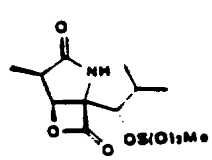

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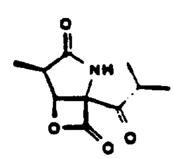

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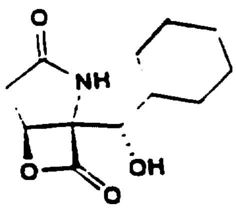
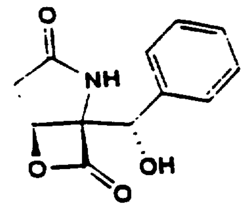


J-1



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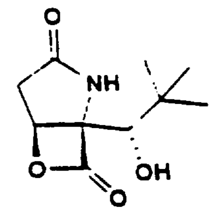

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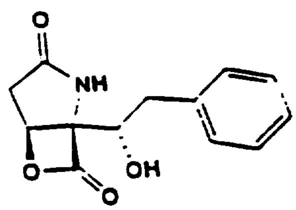

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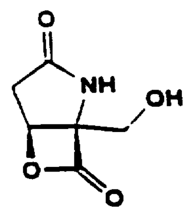

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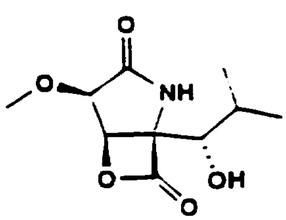

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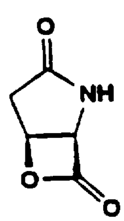

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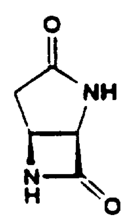
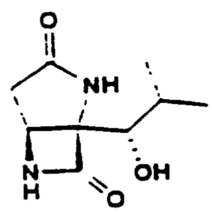
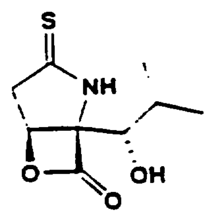


J-8

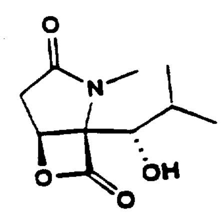
Table C



J-9



J-10



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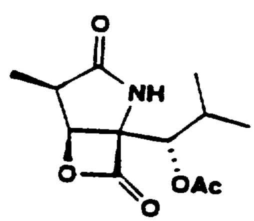
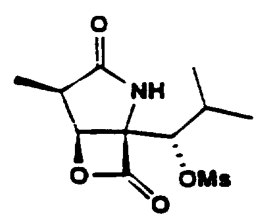


J-12



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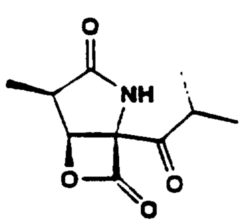

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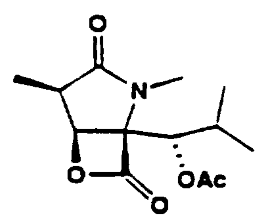


J-15

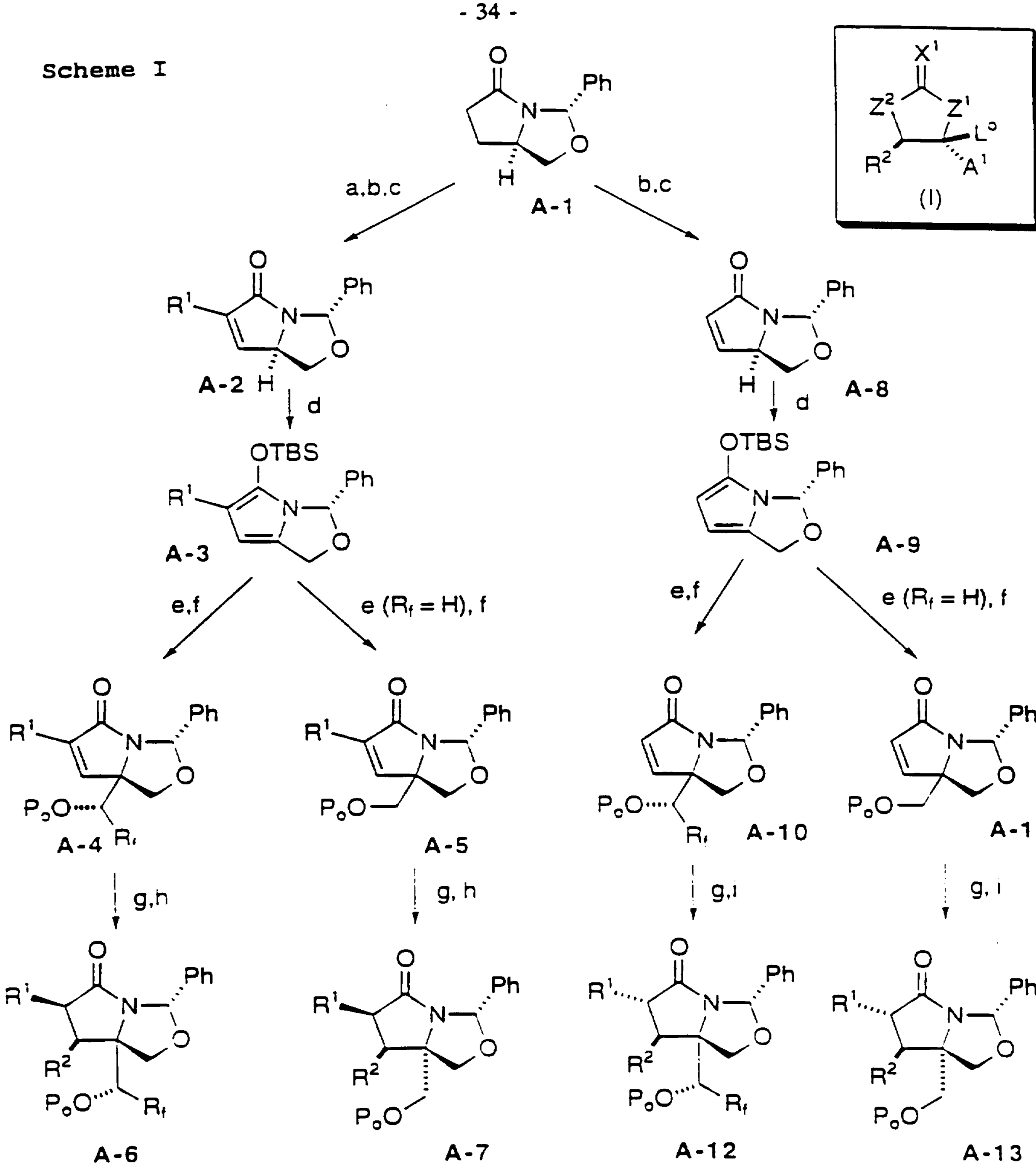
<u>Synthesis</u>

The syntheses disclosed herein are organized by structure groups. A person of skill will recognize that compounds of claim 1 are related to the compounds in the compositions of claim 23, and the compounds of claim 5 are related to the compounds in the compositions of claim 30 and so on. The synthesis of the compounds in claims 1-5 rely primarily on the results reported by Uno, *et al.* in their enantiospecific synthesis of (+)-lactacystin from (*R*)-glutamate [Uno, *et al.*, *J. Am. Chem. Soc.* (1994) 116:2139]. Note that in all schemes relating to claims 1-5 and 24-32, when a straight line is used to connect A^1 (or anything corresponding to A^1) to the rest of the molecule, a dashed line should be assumed. The straight line is used simply for clarity. All A^1 are attached to the rest of the molecule on the "alpha," i.e., the bottom, face.) Compound **A-1** (Scheme 1) serves as the starting material for all synthetic routes proposed in claim 2, for example. Thus, conversion of **A-1** to **A-2**, in analogy to the above-mentioned work allows for introduction of R^1 *via* standard alkylation chemistry. In the cases where R^1 is hydroxyl, halide, $-SR_d$, or $-NR_dR_e$, wherein R_d and R_e each, independently, is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, the electrophile in step b is, respectively, a N-arylsulfonyl-3-phenyloxaziridine [Davis, F.A. *et al.*, *J. Org. Chem.* (1984), 49:3241], a N-halosuccinimide [Stotter, P.A. *et al.*, *J. Org. Chem.* (1973) 38:2576], R_dS-SR_d or elemental sulfur [Zoretic, P.A. *et al.*, *J. Org. Chem.* (1976) 41:3587] [Gassman, P.G. *et al.*, *J. Org. Chem.* (1977) 42:3236], a N-arylsulfonylazide (followed by reduction of the azide to the primary amine and elaboration to NR_dR_e) [Evans, D.A. *et al.*, *J. Am. Chem.* (1987) 109:6881]. If R^1 is labile or reactive, or both, a protecting group would be introduced at this point and

removed at the end of the synthesis. The preparation of compounds of type **A-2** is then completed with the two-step (steps b and c) process reported by Uno [Uno, *et al.*, (1994)].

Introduction of A^1 is the next task. Uno [Uno, *et al.*, (1994)] showed that under Lewis-acid mediated aldol addition conditions, A^1 is introduced with very high facial selectivity with respect to the bicyclic aminoacid derivative **A-2**. This carbon-carbon bond construction allows for the A^1 groups detailed in claim 2. Accordingly, conversion of **A-2** to **A-3**, as described by Uno,
followed by treatment of **A-3** with $SnCl_4$ and an aldehyde (R_fCHO, where R_f corresponds to H or to the rest of A^1), in diethylether yields aldol products **A-4** (or **A-5** if R_f is H), following suitable protection of the hydroxyl group with a group designated here as P_o. [Greene and Wuts, *et al.*, *Protective Groups in Organic Synthesis*, 2nd ed., (1991)].

Scheme I



a) LDA: electrophile b) LDA; PhSeBr c) O_3; pyridine d) TBSOTf, 2,6-lutidine e) R_fCHO, $SnCl_4$, Et_2O f) Protect hydroxyl as P_oO g) Nucleophile corresponding to R^2 h) acidic water i) Electrophile corresponding to R^1

Introduction of R^2 by a stereoselective, conjugate nucleophilic addition to the unsaturated carbonyl system of **A-4** or **A-5**, followed by quenching of the resulting enolate with acidic water gives access to compounds **A-6** and **A-7**, respectively. This process is analogous to the "three-component coupling" strategy for prostaglandin synthesis of [Suzuki, *et al*. (1988)]. In this case, the three components are **A-4** (or **A-5**), R^2, and a proton. The diastereomers shown for **A-6** and **A-7** are predicted to be the major ones based on several facts. The Uno synthesis [Uno, H., et al., (1994)] used an OsO_4-catalyzed dihydroxylation of a compound similar to **A-4** (R^1 = methyl, R_f = *iso*-propyl) to introduce a hydroxyl group corresponding to R^2. This reaction proceeded with complete facial selectivity. Thus, in general, groups with appropriate reactivity (nucleophiles or electrophiles) similarly approach preferentially the "beta" face (from the top) of **A-4** or **A-5**.

Quenching of the enolate with acidic water proceeds stereoselectively. In Suzuki's three-component coupling strategy, [Suzuki, *et al*., (1988)] the nucleophile and electrophile are introduced *trans* to each other. In our case, R^2 and the proton are introduced *trans* to each other. In the event that compounds in which R^1 and R^2 are *trans* to each other are desired, as depicted in compounds **A-12** and **A-13**, a similar strategy has been devised.

The basic strategy has one exception to that detailed above: The order of introduction of R^1 and proton at the carbon *alpha* to the carbonyl is reversed. Thus, conversion of **A-1** to **A-9** is carried out using the same procedures as those for the preparation of **A-3**, omitting the alkylation step (step a). Elaboration of **A-9** to **A-10** and **A-11** utilizes the same conditions as those which afford **A-4** and **A-5**. Addition of R^2 still occurs from the top face, but the quenching step with an

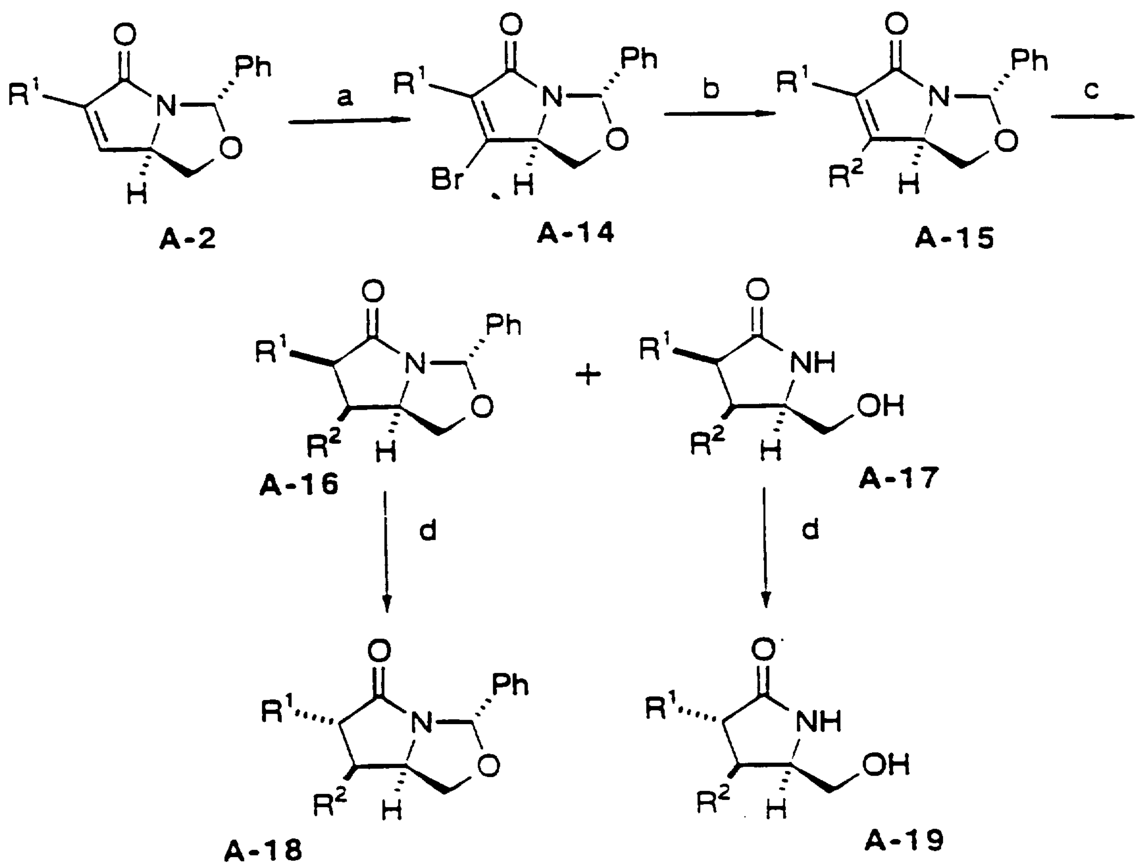
electrophile corresponding to R^1 now gives a *trans* relationship of R^1 and R^2, as shown in compounds **A-12** and **A-13**.

Another advantage of these approaches to **A-6**, **A-7**, **A-12**, and **A-13** is as follows. Should the quenching step introduce the electrophile (proton or R^1) *cis* to R^2 (opposite that predicted above), then one still has stereospecific routes to the desired compounds, except that **A-4** and **A-5** give **A-12** and **A-13**, respectively, and **A-10** and **A-11** give **A-6** and **A-7**.

Compounds in which A^1 is H are prepared by a different strategy (Scheme 2). For example, it has been shown that when A^1 is H, nucleophiles [Hanessian, *et al.*, *Synlett* (1991) 10:222] and electrophiles [Griffart-Brunet, *et al.*, *Tet. Lett.* (1991) 35:119] approach from the bottom face, *cis* to the hydrogen at A^1. Thus, the key to our strategy above is that the stereoselectivity of addition is reversed when A^1 is <u>not</u> H. The rapid preparation described below of compounds in which A^1 is H takes advantage of this reversal of stereoselectivity.

Thus, bromination of **A-2** by standard methods [Caine, *et al.*, *J. Org. Chem.* (1985) 50:2195] gives **A-14**, which is utilized in conjugate addition-elimination procedures to give compounds of type **A-15**. Since A^1 is H, catalytic hydrogenation of the olefin should install R^1 and R^2 in the desired *cis* configuration shown in compound **A-16**. Cleavage of the *N,O*-aminal may occur under such conditions, giving compound **A-17**. Such an occurrence is not disadvantageous since such a deprotection is the next step in the synthesis anyway. Compounds **A-18** and **A-19** are accessed by base-catalyzed epimerization of **A-16** and **A-17**, respectively.

Scheme 2

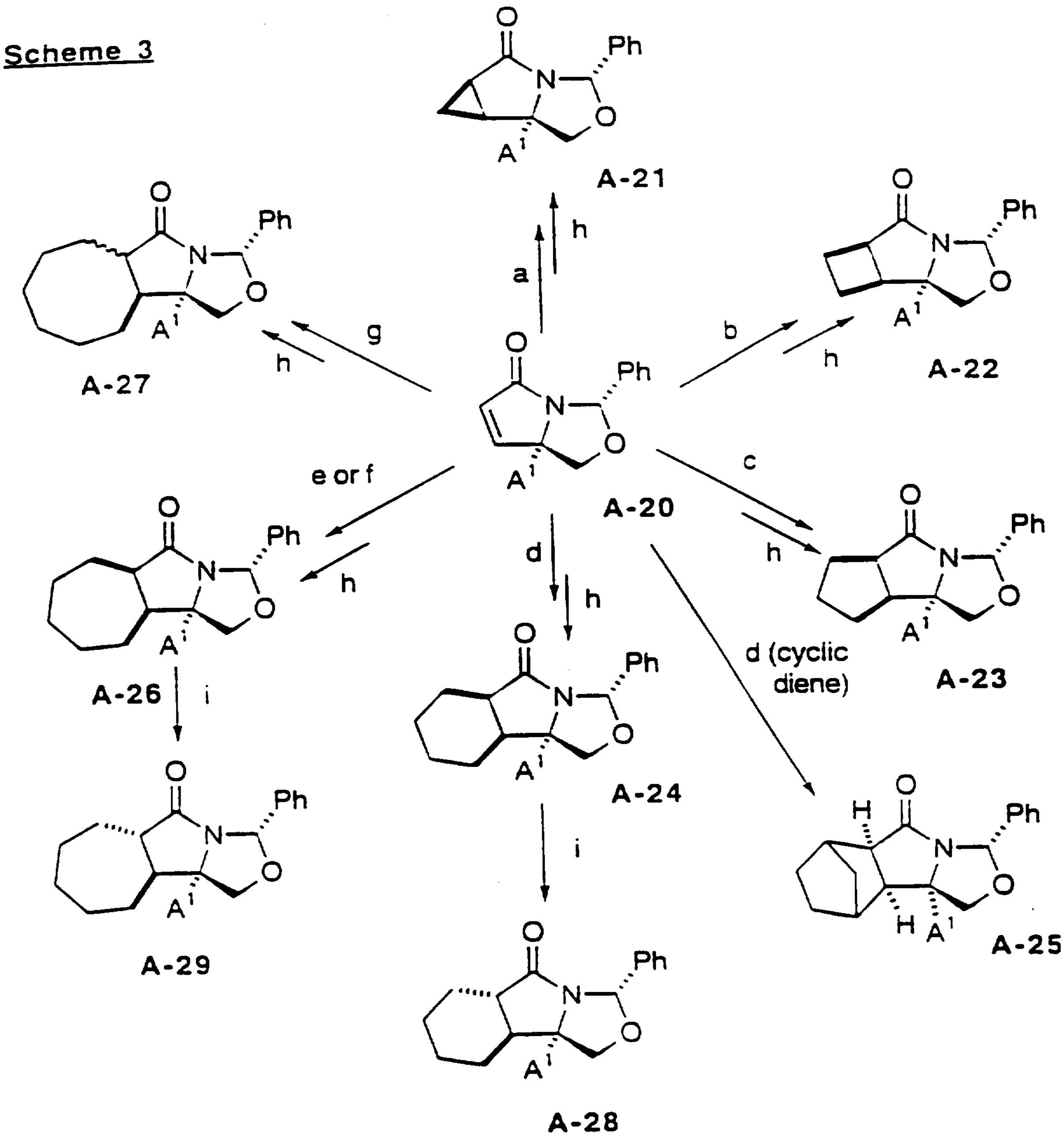


a) Br_2, Et_3N b) Nucleophile corresponding to R^2 ; H_3O^- c) H_2, Pd/C d) DBU, CH_2Cl_2

The above strategies allow for the preparation of compounds containing any and all R^1 and R^2 described in claims 2 and 25, with one exception: compounds where an additional ring is fused to the lactam ring (specifically, compounds in which R^1 is taken together with R^2, giving a bivalent moiety which forms a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl). Their preparation will be discussed below (Schemes 3 and 4). Note that while only carbocyclic systems are depicted in Schemes 3 and 4, both the alicyclic and heterocyclic (aromatic and non-aromatic in both cases) classes of compounds can be prepared in this manner by choice of the appropriate reagents. Further, all substitution patterns on these rings that are normally accessible by such processes are accessible by the methods proposed below. The simplest carbocyclic variants are shown for clarity and to illustrate the general strategy to these compounds.

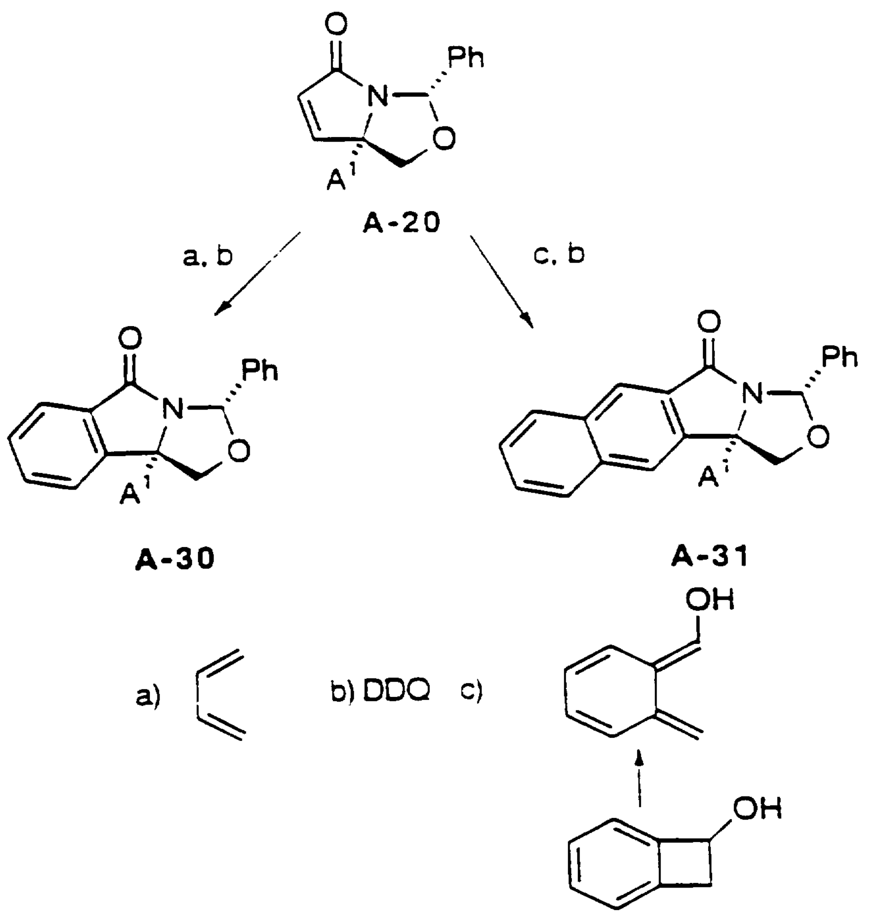
The synthesis of these compounds relies on the diverse chemistry of cycloaddition [Carruthers, *et al.*, *Cycloaddition Reactions in Organic Synthesis* (1990) and J. March, *Advanced Organic Chemistry*, (1992) pp. 826-877] to *alpha-beta* unsaturated olefins. Thus, as shown in Scheme 3, [1+2] processes (e.g. diazoinsertion, cyclopropanation, epoxidation, and aziridination) with compound **A-20** (see Scheme 1 for preparation of compounds of this type, e.g. **A-10** and **A-11**) gives access to compounds of type **A-21**. Compounds of type **A-22** are prepared with a variety of [2+2] cycloaddition processes (e.g. photocycloadditions and ketene additions). 1,3-Dipolar cycloaddition chemistry ([3+2] cycloaddtion reagents, such as nitrile oxides and azomethine ylides) prepares a wide variety compounds of the type **A-23**. The preparation of compounds of type **A-24** is perhaps the most flexible and far-reaching of the cycloaddition chemistry

Scheme 3



a) epoxidation, Simmons-Smith, aziridination, etc. b) photocycloaddition, ketene addition, etc. c) nitrile oxide, azomethine ylides, other [3+2] cycloadditions d) Diels-Alder cycloadditions, etc. e) [5+2] cycloadditions f) LG-$(CH_2)_5$-Nu g) LG-$(CH_2)_6$-Nu h) "functional group manipulation" i) DBU, CH_2Cl_2

Scheme 4



because it relies on the Diels-Alder reaction, one of the most utilized and studied reactions in all of organic chemistry. The variety of compounds of type **A-24** that are prepared by this method is far to great to detail; [Carruthers, (1990)] however, notably, bridged compounds (type **A-25**) are prepared by this method. [5+2] cycloadditions to give **A-26** are less common, but some intriguing examples have been reported. [Wender, *et al.*, *J. Org. Chem.* (1991) 56:6267 and Wender, *et al.*, *Tet. Lett.* (1992) 32:6115].

While all these procedures introduce R^1 and R^2 *cis* to each other, base-catalyzed epimerization in the cases of **A-24** and **A-26** gives access to the *trans*-fused versions of these compounds, **A-28** and **A-29**, respectively. (**A-21**, **A-22**, **A-23**, and **A-25** can not be epimerized in this fashion, as these *trans*-fused rings suffer from much more ring strain than do the *cis*-fused systems.)

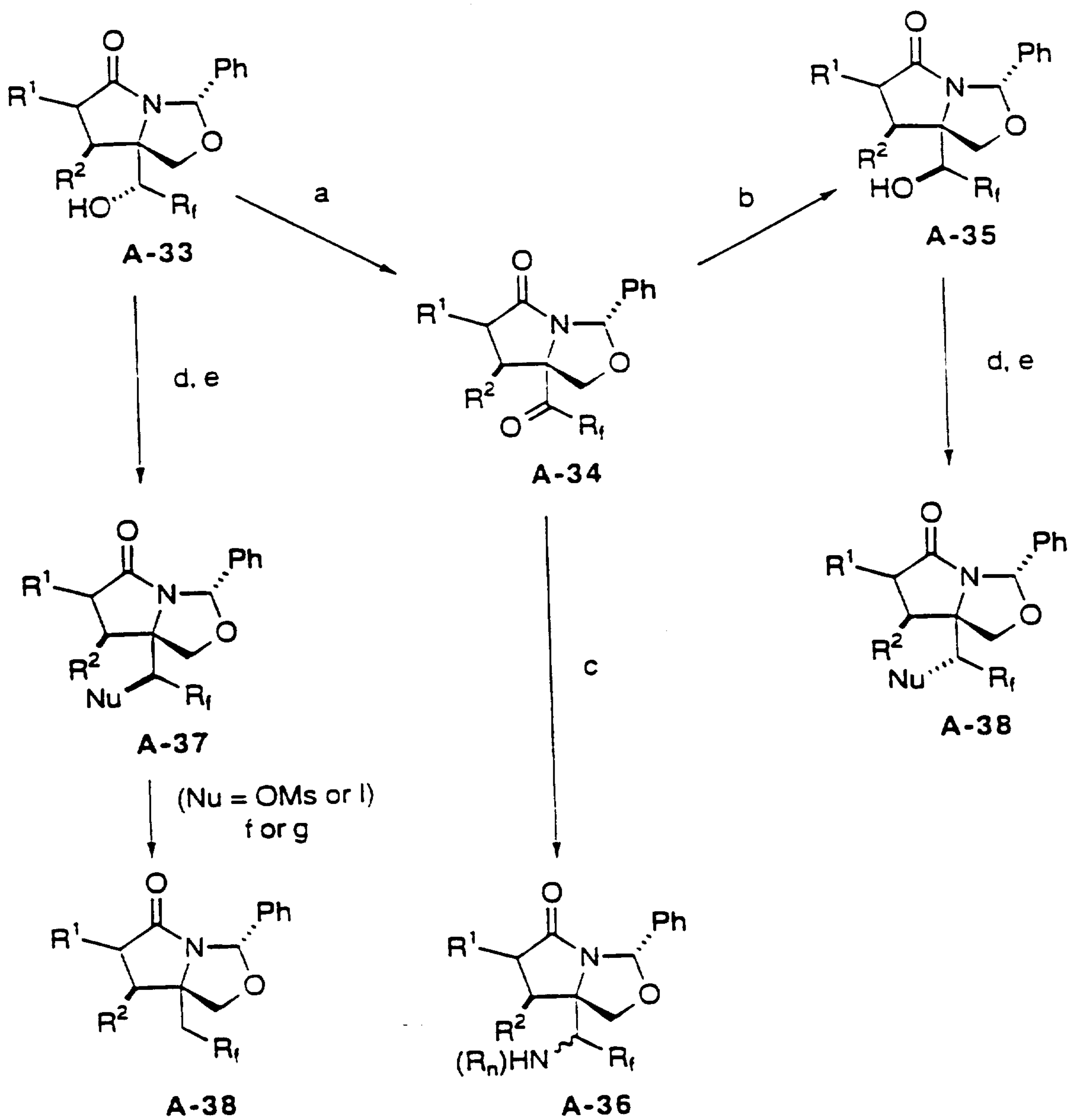
Cycloheptyl (**A-26**) and cyclooctyl (**A-27**) compounds are prepared with a slightly different process, one that is akin to that shown in Scheme 1. Fundamentally, one adds an nucleophile (abbreviated as "Nu") to **A-20** which also has a leaving group (abbreviated as "LG") attached to the other end of the incoming nucleophile, separated by the necessary number of atoms (5 in the case of **A-26**, 6 in the case of **A-27**). Thus, following the addition of the nucleophile, the resulting enolate displaces the leaving group, forming the ring. Alternatively, such a process is done stepwise in order to minimize undesired intramolecular side reactions of the nucleophile and electrophile. These and other methods for making cycloheptyl and cyclooctyl compounds from *alpha-beta* unsaturated carbonyl compounds have been reviewed in the chemical literature. [Petasis, N.A., *et al.*, *Tetrahedron* (1992) 48:5757].

Aryl and heteroaryl compounds (e.g. compounds **A-30** and **A-31**, Scheme 4) are prepared by [4+2] cycloaddition chemistry, followed by oxidation of the resultant cycloalkene or heterocycloalkene with dichlorodicyanoquinone (DDQ). [Pizey, N., *Synth. Reagents* (1977) 3:193-225]. In the case of compounds of type **A-31**, other types of dienes that are utilized are the hydroxyorthoxylylenes, formed by the ring opening reaction of hydroxybenzocyclobutenes. [Arnold, B.J., *et al., J. Chem. Soc.*, (1974) 409-415].

Thus, having prepared all the variations of R^1 and R^2, we turn now to the elaboration of these compounds to include all of the variations of A^1 (Scheme 5).

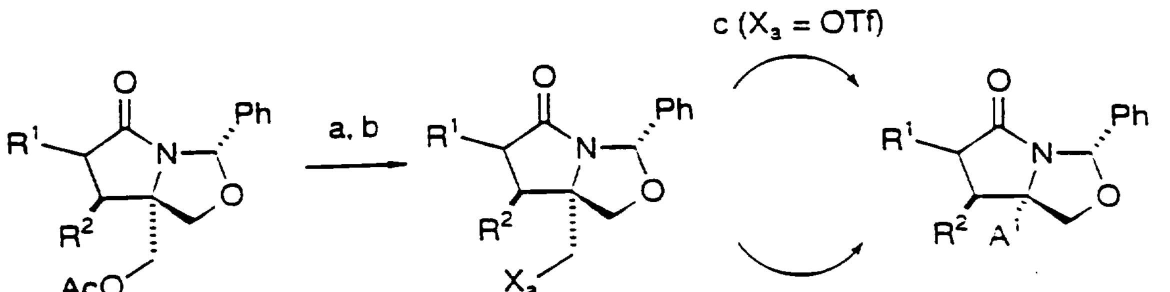
Based on the results of [Uno, *et al.*, (1994)] and as shown in Scheme 1, Lewis-acid catalyzed aldol additions of **A-3** to R_fCHO, followed by further elaboration, provide compounds of the type **A-33** (Scheme 5) with the stereocenters in the configurations shown, including both configurations of R^1. (A straight bond (i.e. neither bold or dashed) is shown here to represent both diastereomers at this position.) Here we will focus on the stereochemistry of the secondary hydroxyl that is included in A^1, and related derivatives. While modification of conditions in the aldol addition (Scheme 1) reaction can provide the other diastereomer at this position, another method of inverting this center is to first oxidize to the ketone under standard conditions (e.g. Swern, Dess-Martin periodinane, etc.) and then reduce with the appropriate reducing agent (e.g. $NaBH_4$, etc.) whose identity is determined by screening a number of reductants. This two-step procedure provides compounds of type **A-35**. Analogously, reductive amination of ketone **A-34** with an amine or hydroxylamine (represented in Scheme 5 as R_nNH_2) and sodium cyanoborohydride ($NaCNBH_3$) provides compounds of type

Scheme 5



a) Dess-Martin periodinane b) $NaBH_4$, e.g. c) R_nNH_2. $NaCNBH_3$
d) MsCl, Et_3N e) Nucleophile f) $LiEt_3BH$ g) Zn, HCl

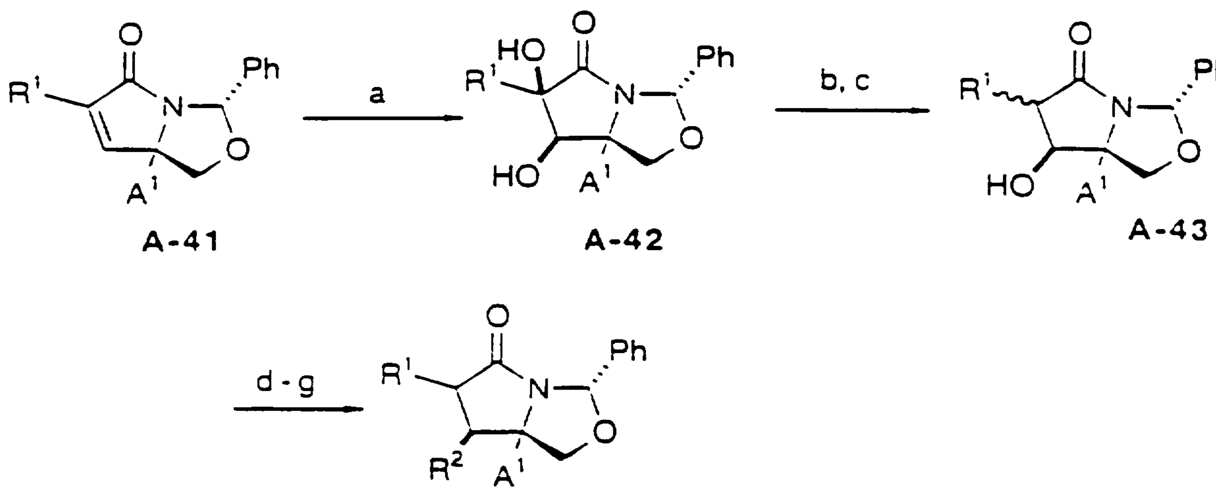
Scheme 6



A-39 A-40 A-38

a) LiOH, THF, H_2O b) Tf_2O, 2,6-di-*tert*-butylpyridine or I_2, PPh_3, imidazole
c) Nucleophile d) metal-halogen exchange; electrophile

Scheme 7



A-44 (subset of A-33)

a) OsO_4, NMO, water, acetone b) TCDI c) Bu_3SnH, AIBN d) separate diastereomers. e) DEAD, Ph_3P, acetic acid f) conversion of acetate to leaving group g) nucleophile corresponding to R^2

A-36. The sense of stereochemical induction in this step can be altered by using a different reducing agent. Alternatively, by first preparing the methanesulfonate ester ("mesylate") of **A-33** or **A-35**, and then displacing the mesylate with a nucleophile (e.g. RNH_2, RS-, RO-, halide, hydroxylamine, Grignard reagents, etc.) one obtains a wide variety of A^1 derivatives with complete control of stereochemistry at the carbon corresponding to the secondary hydroxyl in **A-33**. Reduction of the mesylate (with lithium triethylborohydride ($LiEt_3BH$)) or of a halide (with, e.g., zinc in hydrochloric acid) provides compounds of the type **A-38**.

Another method to produce the various A^1 derivatives is depicted in Scheme 6. Compounds of the type **A-39**, which are prepared according to Scheme 1, are converted to those of type **A-40** where X_a is a halide or trifluoromethanesulfonate ester ("triflate"), for example, using standard functional group manipulation. Displacement of these leaving groups with nucleophiles gives another method for the preparation of compounds of type **A-38**. Subjecting iodide **A-40** (X_a is I) to metal-halogen exchange conditions (e.g. activated Mg metal or *tert*-butyllithium) provides nucleophiles to which a variety of nucleophiles can be added, providing yet another route to compounds of type **A-38**.

Another strategy for the preparation of a subset of compounds of type 33 is worth noting (Scheme 7). This strategy allows for the preparation of compounds of type **A-33** for all R^2 discussed above and for all R^1 other than hydroxyl, halide, $-SR_d$,$-NR_dR_e$. Compounds of the type **A-4**, **A-5**, **A-10**, and **A-11** as shown in Scheme 1 (represented as compound **A-41** in Scheme 7), in which R^1 is attached to the lactam ring with a carbon-carbon bond, can be dihydroxylated to give compounds of type **A-42**, in which the hydroxyl groups have been added to the top face

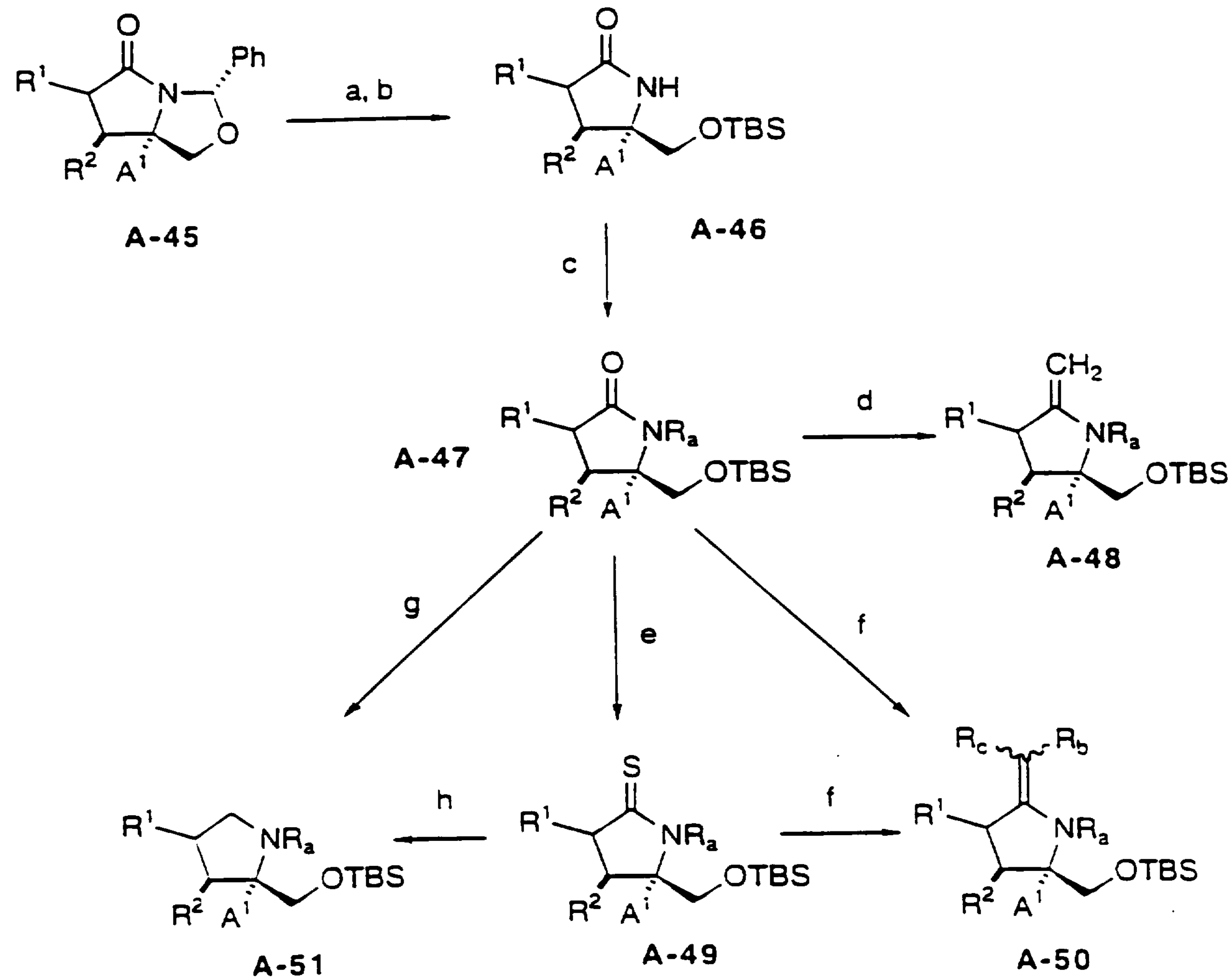
exclusively. [Uno, *et al.*, (1994)] (The reasons for this stereoselectivity have been discussed above.) Following the procedure of [Uno, *et al.*, 1994)] the tertiary hydroxyl can be removed selectively, yielding a mixture of R^1 diastereomers **A-43**, which can be separated by a variety of chromatographic methods. Mitsunobu inversion of the secondary hydroxyl, followed by displacement of a suitably derived leaving group by any nucleophile corresponding to R^2 (e.g. hydroxide, alkoxide, sulfide, Grignard reagents, amine, halide, etc.) gives compounds of the type **A-44**, which are a subset of compounds of the type **A-33**, and can therefore be elaborated into their corresponding A^1 derivatives as described above.

Thus, having provided viable routes to all variations of R^1, R^2, and now A^1 in claims 2 and 25, we now turn our attention to the installation of X^1, L^1, and X^2 (Scheme 8).

The compound of general structure **A-45** can be converted to **A-46** in a two-step process involving first catalytic hydrogenolysis or mild acidic hydrolysis [Corey, E.J., *et al.*, *J. Am. Chem. Soc.* (1992a), 114:10677] and then protection of the primary hydroxyl group as its *tert*-butyldimethylsilyl (TBS) ether under standard conditions. [Corey, E.J. and Venkateswarlu, A., *J. Am. Chem. Soc.* (1972), 94:6190] *N*-Alkylation of the amide provides access to compounds **A-47**. [Challis, N., *The Chemistry of Amides*, (1970) 734-754]. (In the cases where R_b is desired to be H, the amide can be blocked with a suitable protecting group that will be removed at the end of the synthesis. [Greene, T.W., *et al.*, *Protective Groups in Organic Synthesis* (1991)].

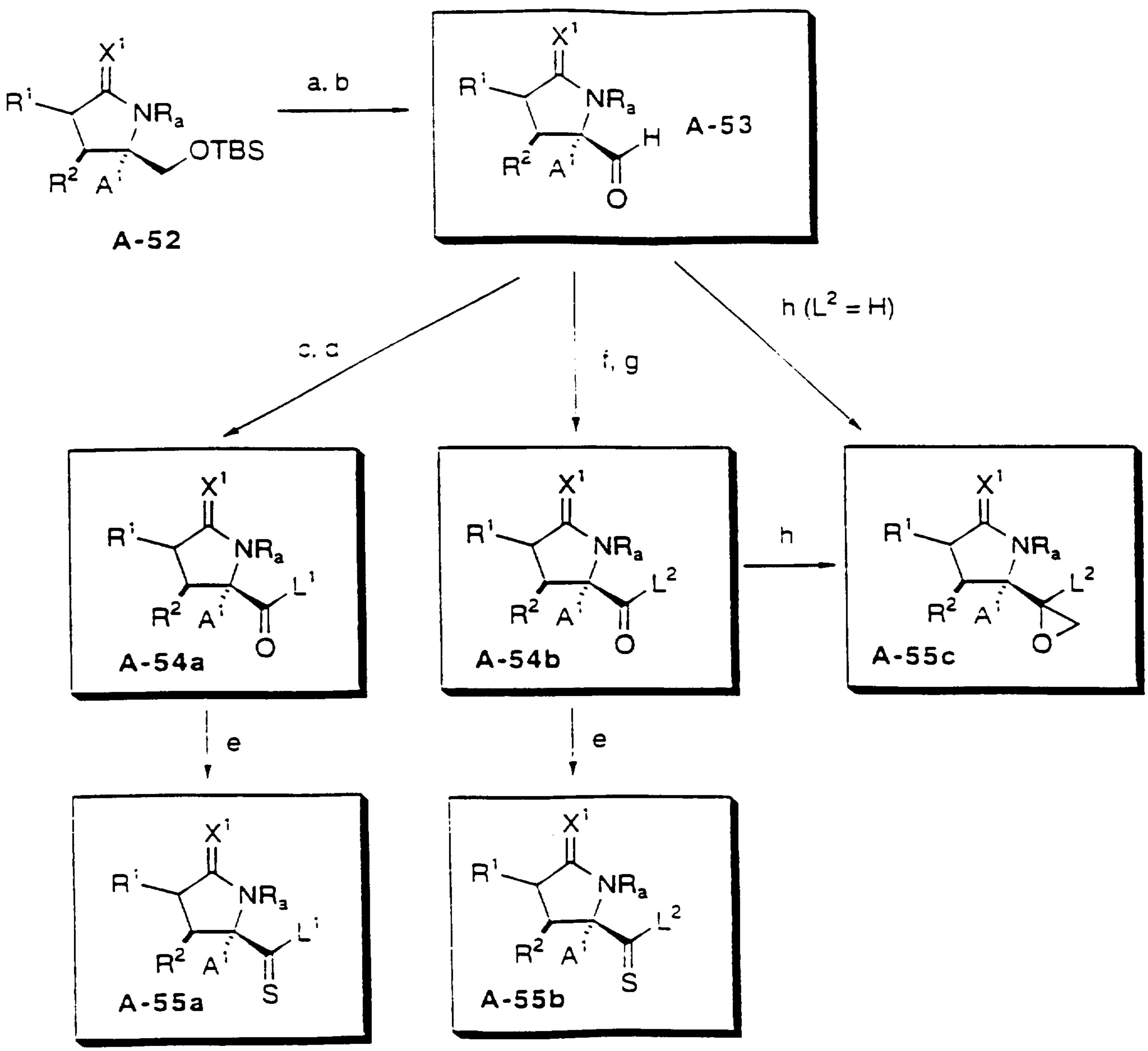
Enamine **A-48** can be prepared from **A-47** under Tebbe olefination conditions. [Pine, S.H., *et al.*, *J. Org. Chem.* (1985) 50:1212]. Thioamide **A-49** can be prepared by treatment of **A-47** with bis(tricyclohexyltin)sulfide and

Scheme 8



a) H_2, Pd/C, H^- or $HS(CH_2)_3SH$, HCl, CF_3CH_2OH b) TBS-Cl, imidazole, DMF
c) NaH, electrophile corresponding to R_a, DMF d) Tebbe olefination
e) $((cyclohexyl)_3Sn)_2S$, BCl_3 f) $R_cR_bC(H)(MgBr)$; H^- g) $LiAlH_4$, Et_2O h) Raney Ni

Scheme 9

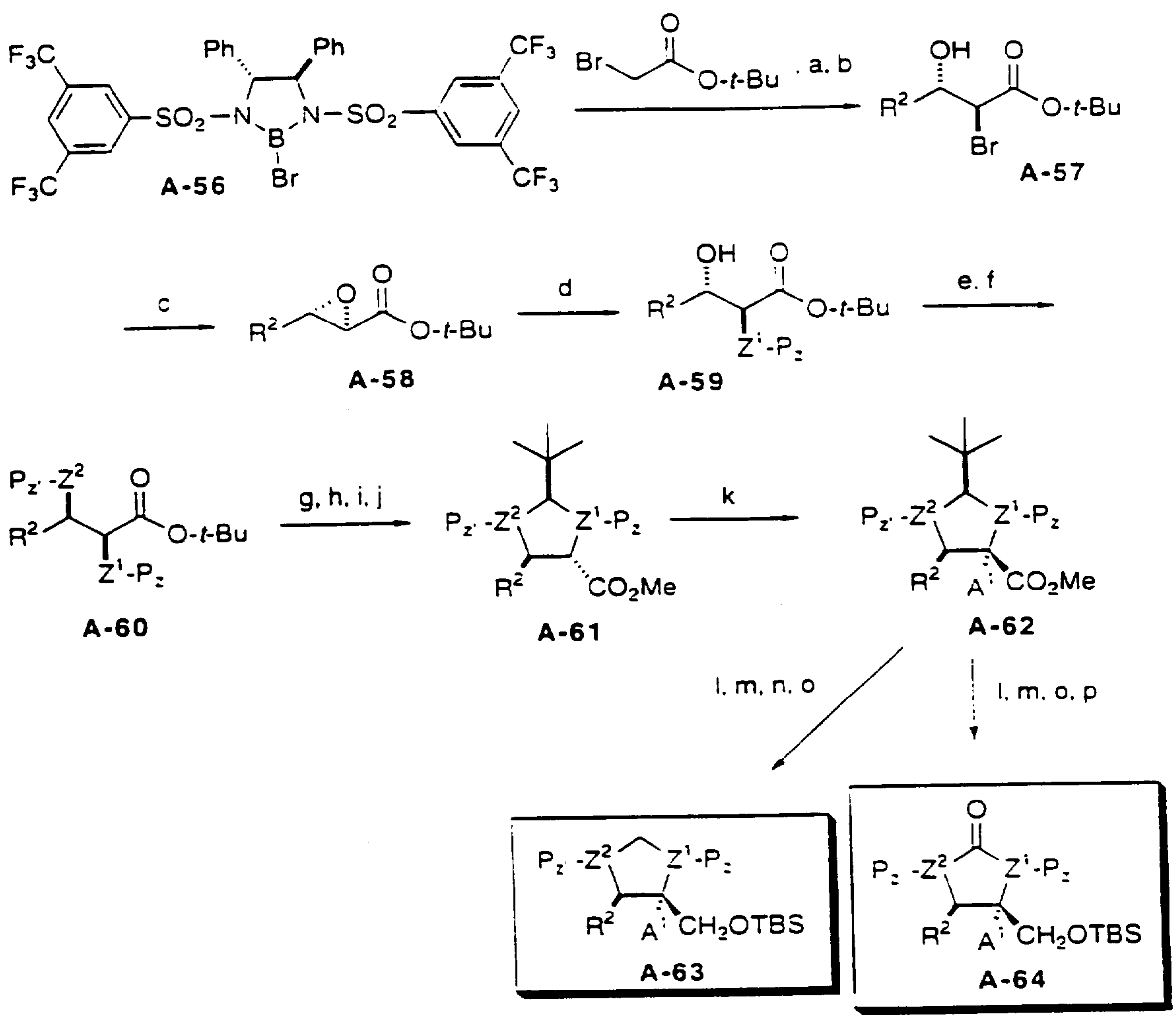


a) TBAF, THF or HF, CH_3CN or 1% HCl, MeOH b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2 c) $NaClO_2$, pH 7, THF d) L^1–H, BOP-Cl e) Lawesson's reagent f) Nucleophile corresponding to L^2 g) Dess-Martin periodinane h) $Me_2(S^+)(O)CH_2^-$

boron trichloride. [Steliou, K., *et al.*, *J. Am. Chem. Soc.*, (1982) 104:3104]. Substituted enamines **A-50** can be prepared by addition of the appropriate Grignard reagent or alkyllithium to **A-47** or **A-49**, followed by acidic hydrolysis, and separation of the E and Z olefin isomers. [Aguerro, A., *et al.*, *J. Chem. Soc.*, *Chem. Commun.* (1986) 531 and Schrock, R.R., *J. Am. Chem. Soc.*, (1976) 98:5399, and Hansen, C., J. *Org. Chem.* (1973) 38:3074]. Pyrrolidines of type **51** can be prepared by reduction of **A-47** with lithium aluminum hydride [March, J., *Advanced Organic Chemistry* (1992) 826-877 and Gaylord, J., *Reduction With Complex Metal Hydrides* (1956)] 544-636] or alternatively by reduction of **A-49** with Raney nickel. [Belen'kii, W., *Chemistry of Organosulfur Compounds* 91990) 193-228].

Taken together, these procedures constitute a synthesis of compounds of the general structure **A-52**, which are converted to **A-53** following, for example, the procedure of Uno, et al. [Uno, H., et al., (1994)] (Scheme 9). Compounds **A-53** are converted to analogues **A-54a** *via* oxidation to the acid and coupling with L^1 (all of which can be prepared by standard methods) under the conditions utilized by Corey and Reichard [Corey, E.J., et al., (1992a]. Sulfurization of these compounds with Lawesson's reagent [Cava, M.P., *et al.*, *Tetrahedron* (1985) 41:5061] gives thiono analogues **A-55a**. Compounds **A-54b** are prepared via addition of a nucleophile (e.g. CF_3) [Francese, C., *et al.*, *J. Chem. Soc.*, *Chem. Commun* (1987) 642], corresponding to L^2 to the aldehydes **A-54b** followed by oxidation of the alcohol with Dess-Martin periodinane. [Linderman, R.J., *et al.*, *Tet. Lett.* (1987) 28:4259]. Sulfurization of these compounds with Lawesson's reagent [Cava, M.P., et al., (1985)] gives thiono analogues **A-55b**. Epoxides of the type **A-55c** are prepared from **A-53** or **A-54b** by the method of Johnson

Scheme 10



a) Et_3N, toluene b) R_iCHO c) K_2CO_3, MeOH, H_2O d) nucleophile corresponding to Z^1; protection of Z^1, if necessary e) TsCl, pyridine f) nucleophile corresponding to Z^2; protection of Z^2, if necessary g) deprotect P_z and $P_{z'}$, if necessary h) $MeSO_3H$, toluene i) CH_2N_2 j) $(CH_3)_3CCHO$, acid k) LiHMDS; electrophile corresponding to A^1 l) $LiAlH_4$ m) TBS-Cl, imidazole, DMF n) acidic water o) HCHO, acid p) CDI

[Johnson, C.R., *Acc. Chem. Res.* (1973) 6:341], thus completing the synthesis of all the analogues detailed in this section.

The preparation of the compounds covered by claims 4 and 31 relies primarily on the methods of Seebach and of Corey. Thus, as shown in Scheme 10, epoxides of the type **A-58** are prepared according to the procedure of Corey, *et al.*, [Corey, E.J., et al., (1992b)] from **61** *via* compound **62**. R^2 is thus installed by choosing the appropriate aldehyde for the aldol reaction in step a. Corey [Corey, E.J., et al., (1992b)] showed that epoxides **A-58** are opened stereospecifically to give **A-59**. Thus, Z^1 is installed by treating **A-58** with a nucleophile corresponding to Z^1. (P_z refers to a protecting group for Z^1.) Installation of Z^2 is accomplished by conversion of the hydroxyl **A-59** to a leaving group, for example, a tosylate, and displacement of the leaving group with a nucleophile corresponding to Z^2. (P_z, refers to a protecting group for Z^2).

The results of Seebach [Seebach, D., *et al., Helv. Chim. Acta.* (1987) 70:1194] are used in the next part of the synthesis. Conversion of **A-60** to **A-61** allows for enolization and alkylation with an electrophile corresponding to A^1, yielding **A-62**. Seebach showed that such alkylations give predominantly the diastereomer shown.

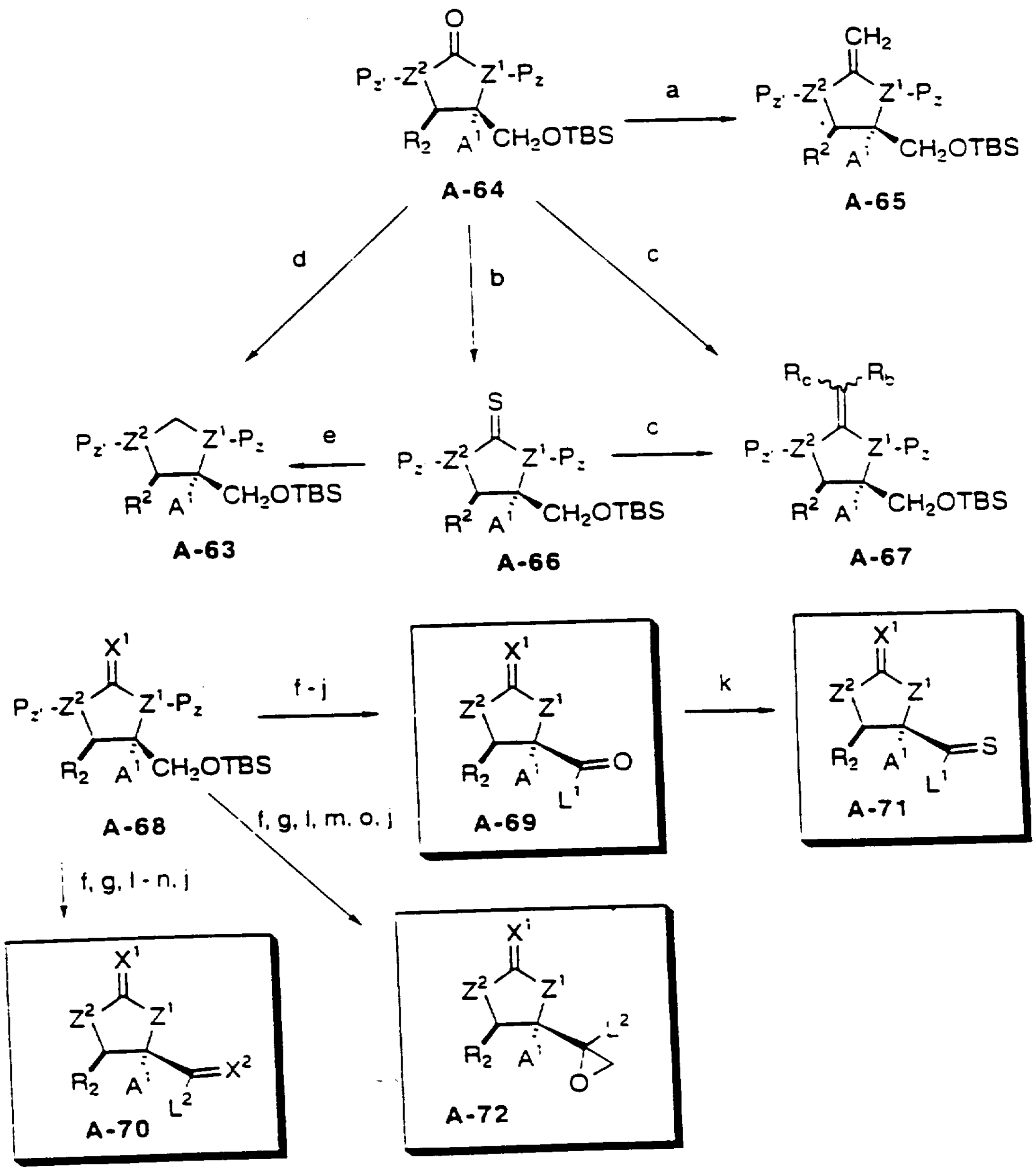
After reduction of the ester and protection of the resulting primary alcohol as the TBS ether, removal of the *tert*-butylmethylene protecting group, enables conversion to compounds of either the type **A-63** (by treatment with, for example, formaldehyde and an acid catalyst) or the type **A-64** (by treatment with, for example, CDI) is effected.

Compound is a key intermediate in the completion of thesynthesis of the compounds in claims 4 and 31, (Scheme

11). Compounds of the type **A-65** are prepared from **A-64** under Tebbe olefination conditions. [Pine, S.H., et al., (1985)]. Compounds of the type **A-66** are prepared by treatment of **A-64** with bis(tricyclohexyltin)sulfide and boron trichloride. [Steliou, K., et al., (1982)]. Compounds of the type **A-67** are prepared by addition of the appropriate Grigard reagent or alkyllithium to **A-64** or **A-66**, followed by acidic hydrolysis, and separation of the E and Z olefin isomers. [Aguerro, A., et al., (1986) and Schrock, R.R., (1976), and Hansen, C., et al., (1973)]. Compounds of the type **A-63**, prepared in Scheme 10, are also prepared by reduction of **A-64** with lithium aluminum hydride [March, J., (1992), and Gaylord, J. (1956)] or alternatively by reduction of **A-66** with Raney nickel. [Belen'kii, W., (1990)].

Taken together, compounds of the type **A-63**, **A-64**, **A-65**, **A-66**, and **A-67** are of the general class **A-68**, which are converted the general class of lactacystin analogues **A-69** by fluoride deprotection of the TBS ether, oxidation of the resulting primary alcohol to the carboxylic acid, *via* the aldehyde analogues, in, for example, the two-step process shown, and coupling with L^1 using the method of Corey and Reichard, [Corey, E.J., et al., (1992a)] and removal of the protecting groups on Z^1 and Z^2 (if necessary). Lactacystin analogues **A-71** are prepared by treating **A-69** with Lawesson's reagent [Cava, M.P., et al., (1985)]. Analogues **A-70** are also prepared from **A-68** by fluoride deprotection of the TBS ether, oxidation of the resulting primary alcohol to the aldehyde, addition of nucleophile (e.g., CF_3) [Francese, C. et al. 1987, supra] Dess-Martin periodinane oxidation, sulfurization with Lawesson's reagent (if desired) [Cava, M.P., 91985)], and deprotection of Z^2 and Z^2, if necessary. Epoxides of the type **A-72** are also prepared from **A-68** by fluoride deprotection of the TBS ether, oxidation of the

Scheme 11

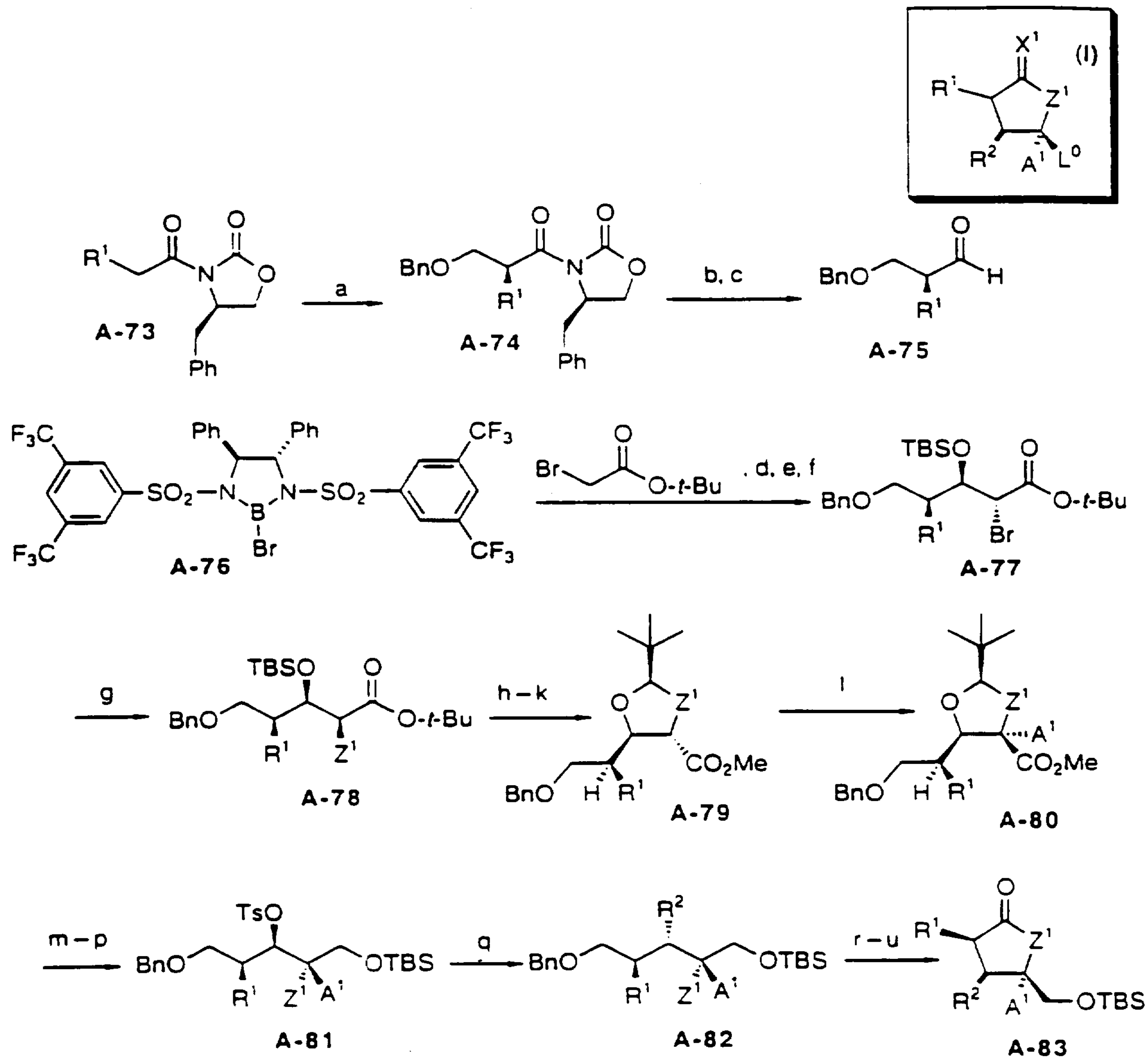


a) Tebbe olefination b) $((cyclohexyl)_3Sn)_2S$, BCl_3 c) $R_cR_bC(H)(MgBr)$; H^- d) $LiAlH_4$, Et_2O e) Raney Ni f) TBAF, THF g) Swern oxidation h) $NaClO_2$, Na_2HPO_4 i) L^1–H, BOP-Cl j) deprotection, if necessary k) Lawesson's reagent l) Nucleophile corresponding to L^2 m) Dess-Martin periodinane n) Step k, if desired o) $Me_2(S^+)(O)CH_2^-$

resulting primary alcohol to the aldehyde, addition of a nucleophile corresponding to L^2, Dess-Martin periodinane oxidation, addition of dimethyloxosulfonium methylide, and deprotection of Z^1 and Z^2 (if necessary). Thus, the above strategy prepares all the compounds in claims 4 and 31.

The preparation of the compounds covered by claim 1 relies primarily on the methods of Evans and of Corey. Thus, as shown in Scheme 12, chiral oxazolidinones of the type **A-73** are enolized and alkylated with benzyloxy-bromomethane, thereby installing R^1. [Evans, D.A., *et al. J. Am. Chem. Soc.*, (1982) 104:1737]. (Only one configuration of R^1 is obtained in the reaction. The other configuration of R^1 is obtained by using the opposite enantiomer of the chiral oxazolidinone.) Conversion of **A-74** to aldehydes of the type **A-75**, and chiral, boron-mediated (using **A-76**) aldol condensation with *tert*-butylbromoacetate gives [Corey, E.J., et al., (1992b)] a secondary alcohol that is protected as the *tert*-butyldimethylsilyl (TBS) ether to give compounds of the type **A-77**. Displacement of the bromide with a nucleophile [Corey, E.J., et al. (1992b)] corresponding to Z^1 yields **A-78**, which can be converted to compounds of the type **A-79**. As discussed above regarding claim 4, such compounds can be enolized and alkylated stereospecifically with electrophiles corresponding to A^1, giving **A-80**. Standard functional group manipulation yields tosylates of the type **A-81**. Displacement of the tosylate with a nucleophile corresponding to R^2, [Hanessian, S., *et al., J. Org. Chem.* (1989) 54:5831] gives compounds of the type **A-82**, the primary alcohol of which is deprotected *via*, for example, catalytic hydrogenolysis, and cyclized to compounds **A-83** after oxidation of the primary alcohol to the carboxylic acid.

Scheme 12

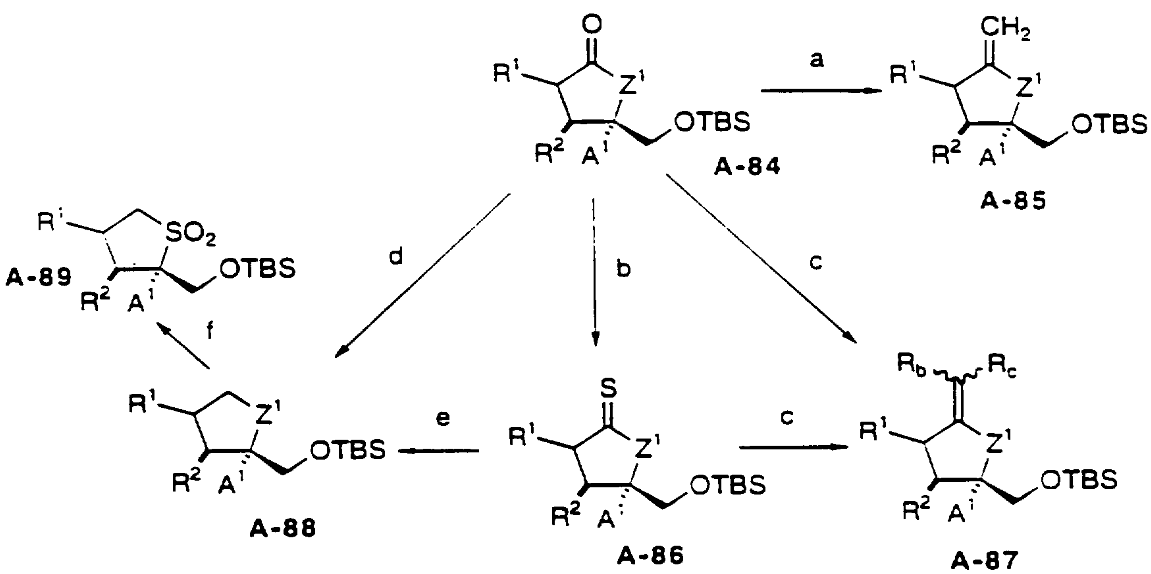


a) LDA: $BnOCH_2Br$ b) $LiAlH_4$ c) Swern Oxidation d) Et_3N, toluene e) **A-75** f) TBS-Cl, imidazole, DMF g) Nucleophile corresponding to Z^1 h) $MeSO_3H$ i) CH_2N_2 j) TBAF, THF k) $(CH_3)_3CCHO$, acid catalyst l) LiHMDS, electrophile corresponding to A^1 m) $LiAlH_4$ n) TBS-Cl, imidazole, DMF o) deprotection p) TsCl, pyridine q) Nucleophile corresponding to R^2 r) H_2, Pd/C s) oxidation t) deprotect Z^1, if necessary u) cyclize (with DCC, for example)

As shown in Scheme 13, compounds of the type **A-84**, in which R^1 is in either the (*R*) or (*S*) configuration, are converted into a variety of X^1 variants. Compounds of the type **A-85** are prepared from **A-84** under Tebbe olefination conditions. [Pine, et al., (1985)] Compounds of the type **A-86** are prepared by treatment of **A-84** with bis(tricyclohexyltin)sulfide and boron trichloride. [Stelious, K., et al., (1982)]. Compounds of the type **A-87** are prepared by addition of the appropriate Grignard reagent or alkyllithium to **A-84** or **A-86** followed by acidic hydrolysis, and separation of the E and Z olefin isomers. [Aguerro, A., et al., (1986), Schrock, R.R., (1976) and Hansen C., et al., (1973)]. Compounds of the type **A-88** are prepared by reduction of **A-84** with lithium aluminum hydride [March, J. (1992), and Gaylord, J., (1956)] or alternatively by reduction of **A-86** with Raney nickel. [Belen'kii, W., (1990)]. When Z^1 is S in **A-88**, oxidation to the cyclic sulfone variants is effected with, for example, $KHSO_5$. [Trost, B.M., *et al., Tet. Lett.* (1981) 22:1287].

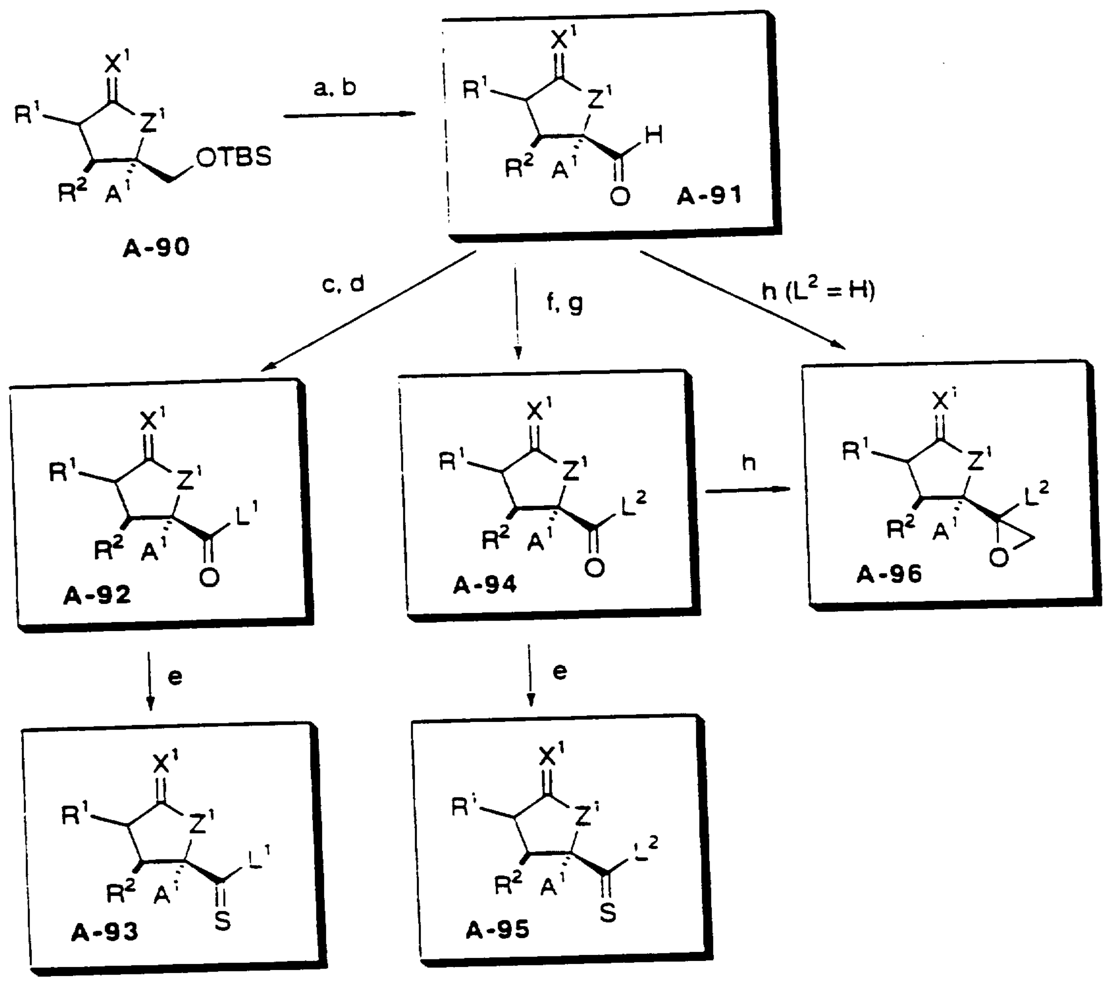
Taken together, compounds of the type **A-84**, **A-85**, **A-86**, **A-87**, **A-88**, and **A-89** are of the general class **A-90**, which are converted analogues **A-91**, following for example, the procedure of Uno, et al. [Uno, H., et al., (1994)] (Scheme 13b). Compounds **A-91** are converted to analogues **A-92** via oxidation to the acid and coupling with L^1 (all of which can be prepared by standard methods) under the conditions utilized by Corey and Reichard. [Corey, E.J., et al., (1992a)]. Sulfurization of these compounds with Lawesson's reagent gives thiono analogues **A-93**. Compounds **A-94** are prepared *via* addition of a nucleophile (e.g. CF_3) [Francese, C., et al., (1987)] corresponding to L^2 to the aldehydes **A-91** followed by oxidation of the alcohol with Dess-Martin periodinane. Sulfurization of these compounds

Scheme 13a



a) Tebbe olefination b) ((cyclohexyl)$_3$Sn)$_2$S. BCl$_3$ c) $R_bR_cC(H)(MgBr)$: H^- d) LiAlH$_4$. Et$_2$O e) Raney Ni f) KHSO$_5$ g) TBAF. THF h) Swern oxidation i) NaClO$_2$. NaH$_2$PO$_4$ j) L^1–H. BOP-Cl k) Lawesson's reagent

Scheme 13b



a) TBAF, THF or HF, CH_3CN or 1% HCl, MeOH b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2 c) $NaClO_2$, pH 7, THF d) L^1–H, BOP-Cl e) Lawesson's reagent f) Nucleophile corresponding to L^2 g) Dess-Martin periodinane h) $Me_2(S^-)(O)CH_2^-$

with Lawesson's reagent gives thiono analogues **A-95**. Epoxides of the type **A-96** are prepared from **A-91** or **A-94** by the method of Johnson, thus completing the synthesis of all the analogues detailed in this section. Note: As above in all Schemes relating to claim 6, when a straight line is used to connect A^2 (or anything corresponding to A^2) to the rest of the molecule, a dashed line should be assumed. The straight line is used simply for clarity. All A^2 are attached to the rest of the molecule on the "alpha" face (i.e. the bottom face).

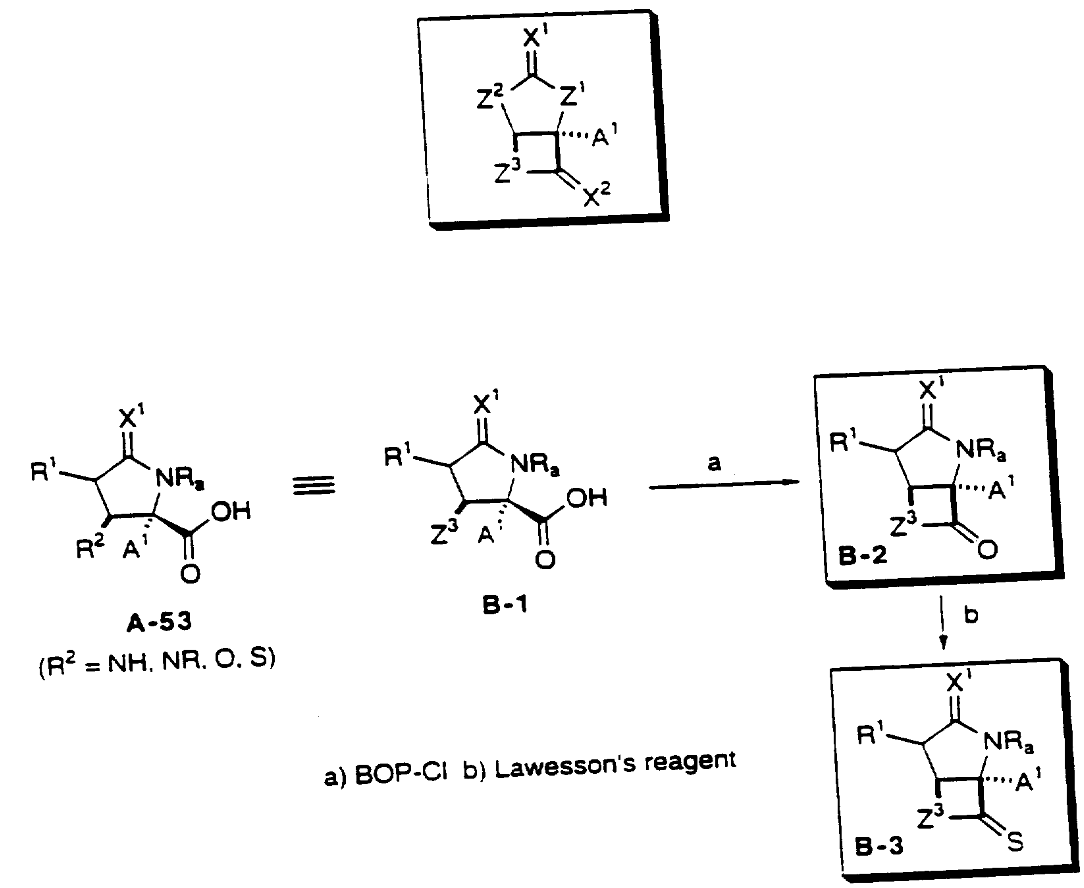
The proposed syntheses of all of the compounds listed in claim 17 rely on a key intermediate from Scheme 9. As shown in Scheme 14, compounds of type **B-1** are analogous to a subset of compounds **A-53**, whose various preparations were described in Schemes 1, 2, and 5-8, and described in the accompanying text.

In order to prepare analog **B-2**, the method of Corey, *et al.* is utilized. [Corey, E.J., *et al., Tet. Lett.* (1993) 34:6977]. Sulfurization of **B-2** with, for example, Lawesson's reagent [Cava, M.P., et al., (1985)] provides analogs **B-3**.

The preparation of compounds of type **B-1** is summarized in Scheme 15. Thus the same starting material (**A-1**) as that used in Scheme 1 is elaborated to all of the compounds **B-2** by the same methods as those used in Schemes 1, 2, 5, 6, and 8. There are then two possible general strategies for the conversion of these intermediates to **B-2**. The first is analogous to that detailed in Schemes 1, 2, 5, 6, 8, and 9, in which Z^5 (suitably activated or protected) serves as the nucleophile in the relevant reactions.

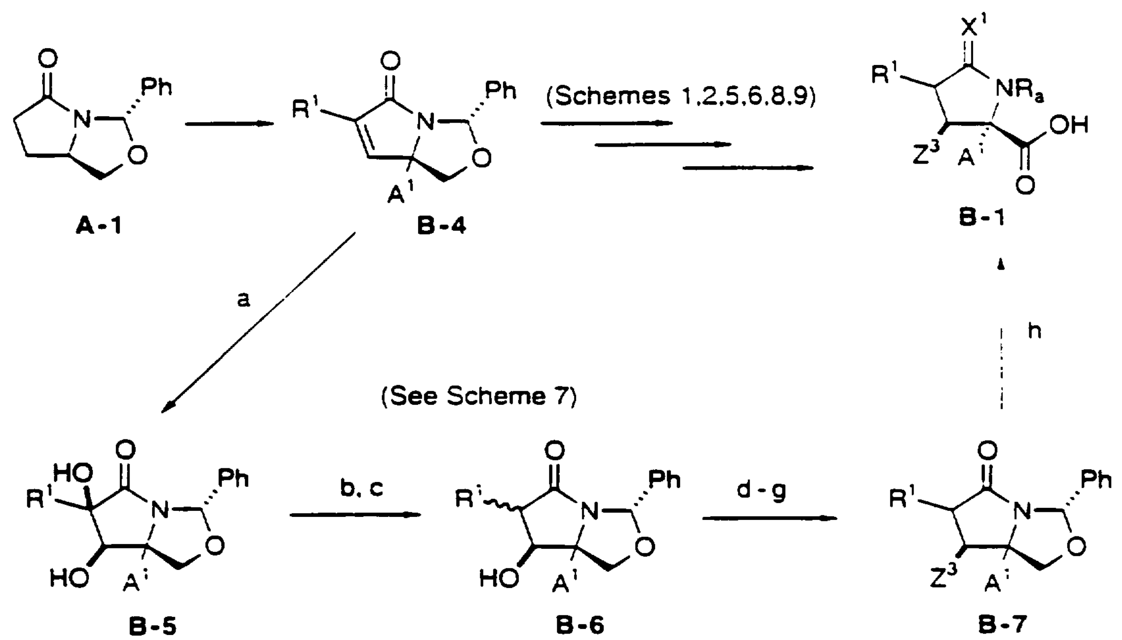
The other strategy, based on that shown in Scheme 7 is also depicted in Scheme 15. Thus, dihydroxylation of **B-4** gives **B-5**, which is then be deoxygenated as before, giving **B-6**. Separation of the diastereomers, Mitsunobu

Scheme 14



a) BOP-Cl b) Lawesson's reagent

Scheme 15



a) OsO_4, NMO, water, acetone b) TCDI c) Bu_3SnH, AIBN d) separate diastereomers e) DEAD, PPh_3, acetic acid f) conversion of acetate to leaving group g) nucleophile corresponding to Z^3. h) see Schemes 8 and 9

inversion of the secondary hydroxyl, and displacement of a leaving group derived from the Mitsunobu product provides compounds of the type **B-7**, which are then elaborated to **B-1** as described in Schemes 8 and 9.

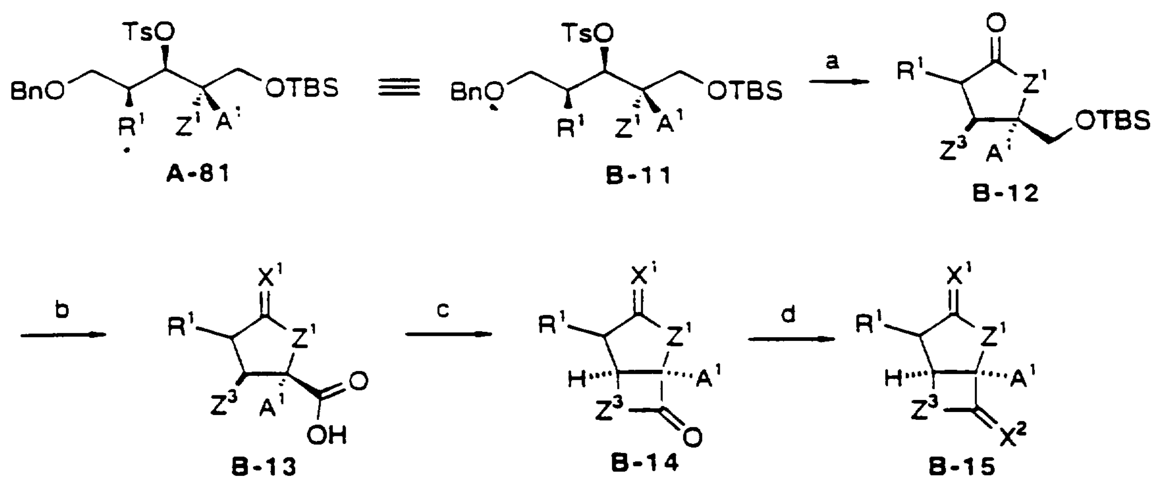
The proposed syntheses of all of the compounds listed in claim 17 rely on intermediate **A-81** from Scheme 12. As shown in Scheme 16, compounds of type **B-11** are analogous to a subset of compounds **A-81**, whose various preparations are illustrated in Scheme 12, and described in the accompanying text. Conversion of compounds of the type **B-11** to **B-12** is performed in analogy to Scheme 12. Compounds of the type **B-13** are prepared from **B-12** following the relevant steps in Schemes 13a and 13b.

In order to prepare analogs **B-14**, the method of Corey, et al. is utilized. [Corey, E.J., et al., (1993)] Sulfurization of **B-14** with, for example, Lawesson's reagent [Cava, M.P., et al., (1985)] provides analogs **B-15**.

The preparation of the compounds covered by claim 7 relies on a key intermediate from Scheme 7. As shown in Scheme 17, compound **C-1** is analogous to **A-41**, whose preparation is illustrated in Scheme 7 and described in the accompanying text. Following the work of Fuchs, [Hutchinson, D.K., *et al., J. Am. Chem. Soc.* (1987)] **C-1** is treated sequentially with bis(benzyloxymethyl)lithium cuprate and acidic water, giving compounds of the type **C-2**. Both configurations of R^7 can be accessed. See Schemes 1 and 2 for details. As in Scheme 8, mild acidic hydrolysis, protection of the primary hydroxyl, and alkylation or protection of the nitrogen gives compounds **C-2**.

Deprotection of the benzyl group by, for example, catalytic hydrogenolysis followed by displacement of the derived tosylate with a nucleophile corresponding to Z^7, accesses compounds **C-3**, in which Z^7 is protected, if

Scheme 16



a) See Scheme 12 b) See Scheme 13b c) BOP-Cl d) Lawesson's reagent

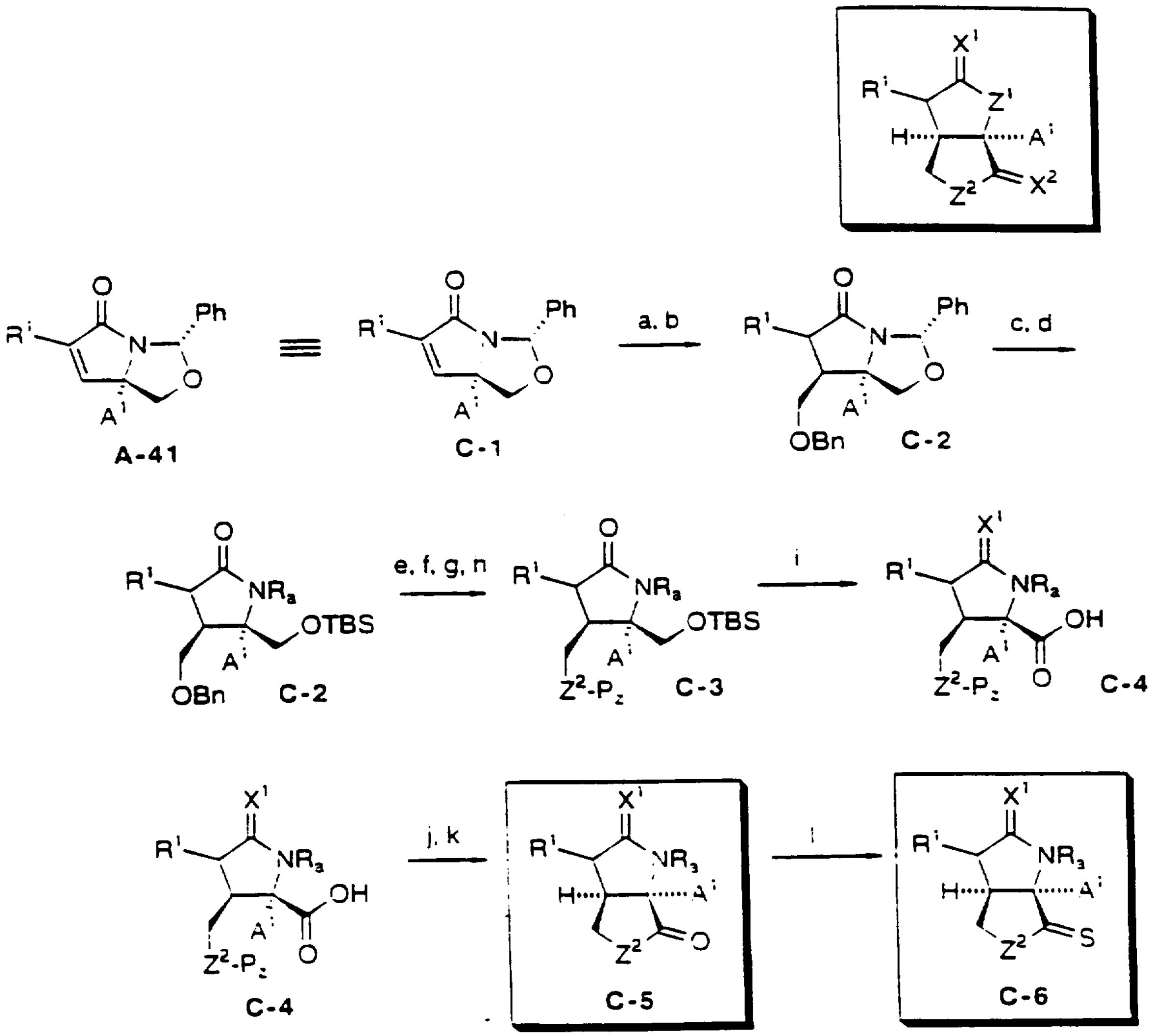
necessary. Elaboration to carboxylic acids **C-4** follows the same set of reactions in Schemes 8 and 9, allowing the preparation of all X^9 variants. Deprotection of Z^7, if necessary, followed by cyclization with, for example, DCC, yields bicyclic structures **C-5** which if desired are sulfurized with Lawesson's reagent [Cava, M.P., et al., (1985)] to give **C-6**.

The preparation of the compounds covered by the right-hand structure in claim 7 relies on a key intermediate from Scheme 9. As shown in Scheme 18, compound **C-11** is analogous to a subset, defined by Z^7, of the class of compounds **A-52**, whose preparation is illustrated in Schemes 1-8, and described in the accompanying text. Deprotection of the primary hydroxyl with, for example, TBAF in THF and oxidation *via*, for example, under Swern oxidation conditions to the aldehydes **C-12**. Homologation of the aldehyde with, for example, the phosphorous ylide derived from triphenylmethoxymethylphosphonium chloride [Jamison, T.F., *et al., J. Am. Chem. Soc.*, (1994) 116:5505] yields enolethers of the type **C-13**, which are converted to carboxylic acids **C-14** with acidic hydrolysis and oxidation of the resulting aldehyde with, for example, buffered $NaClO_2$ oxidation. [Corey, E.J., et al., (1992a)].

Deprotection of Z^7, if necessary, followed by cyclization with, for example, DCC, [Klausner, Y.S. *et al., Synthesis* (1972) 453] yields bicyclic structures **C-15** which if desired are sulfurized with Lawesson's reagent [Cava, M.P., (1985)] to give **C-16**.

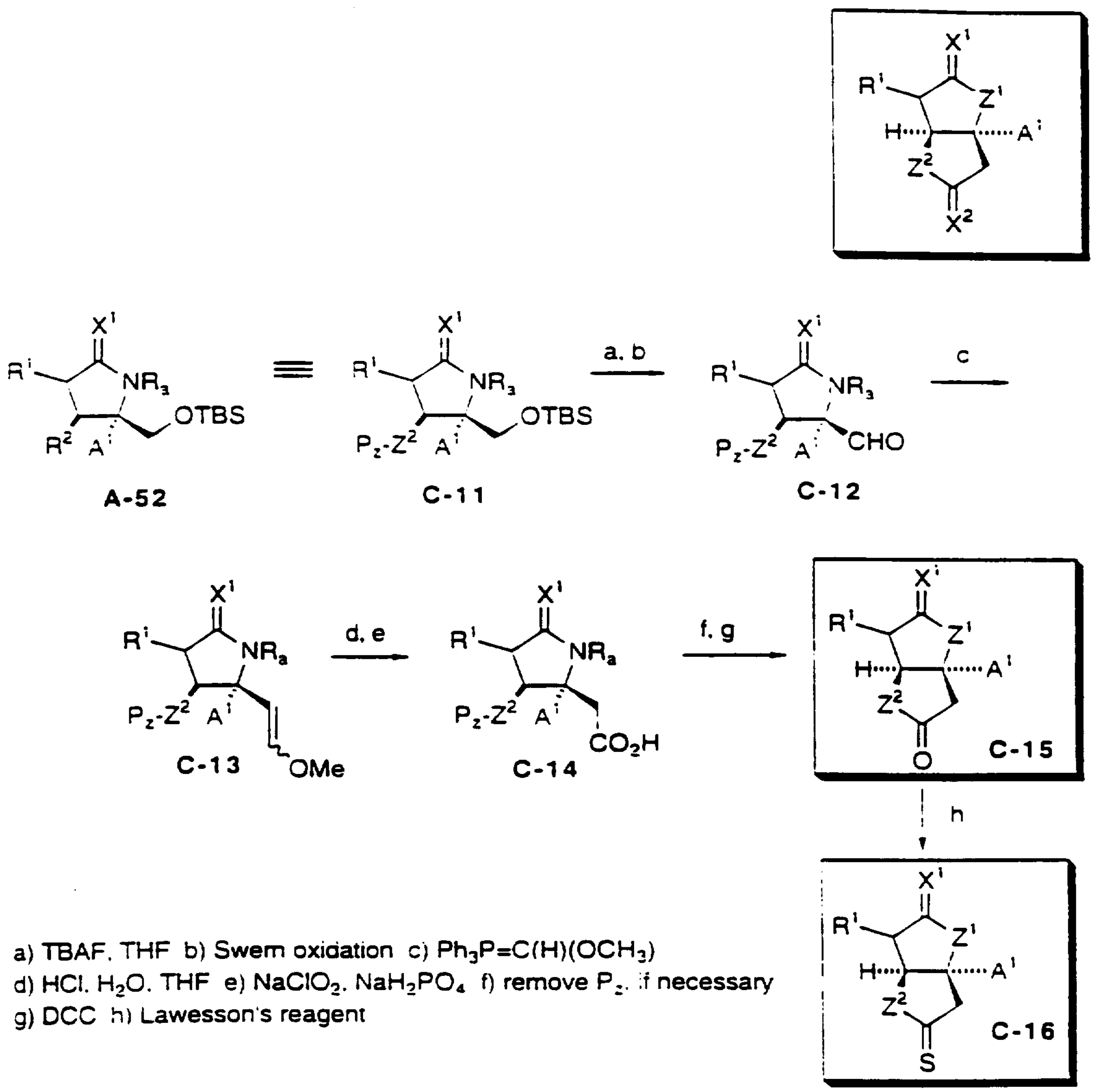
The preparation of the compounds covered by claims 8 and 39 relies primarily on the boron-enolate mediated asymmetric aldol procedures of Evans [Evans, D.A., et al., *J. Am. Chem. Soc.*, (1981) 103:2127] and on the results of studies on intramolecular radical

Scheme 17



a) $(BnOCH_2)_2CuLi$; acidic water b) See also Scheme 1 for cases in which A^1 is H.
c) HCl, $HS(CH_2)_3SH$, CF_3CH_2OH d) See Scheme 8 e) H_2, Pd/C f) TsCl, pyridine
g) Nucleophile corresponding to Z^2 h) Protection of Z^2, if necessary i) See Schemes 8 and 9
j) deprotection of Z^2, if necessary k) DCC l) Lawesson's reagent

Scheme 18



a) TBAF, THF b) Swern oxidation c) $Ph_3P{=}C(H)(OCH_3)$
d) HCl, H_2O, THF e) $NaClO_2$, NaH_2PO_4 f) remove P_z, if necessary
g) DCC h) Lawesson's reagent

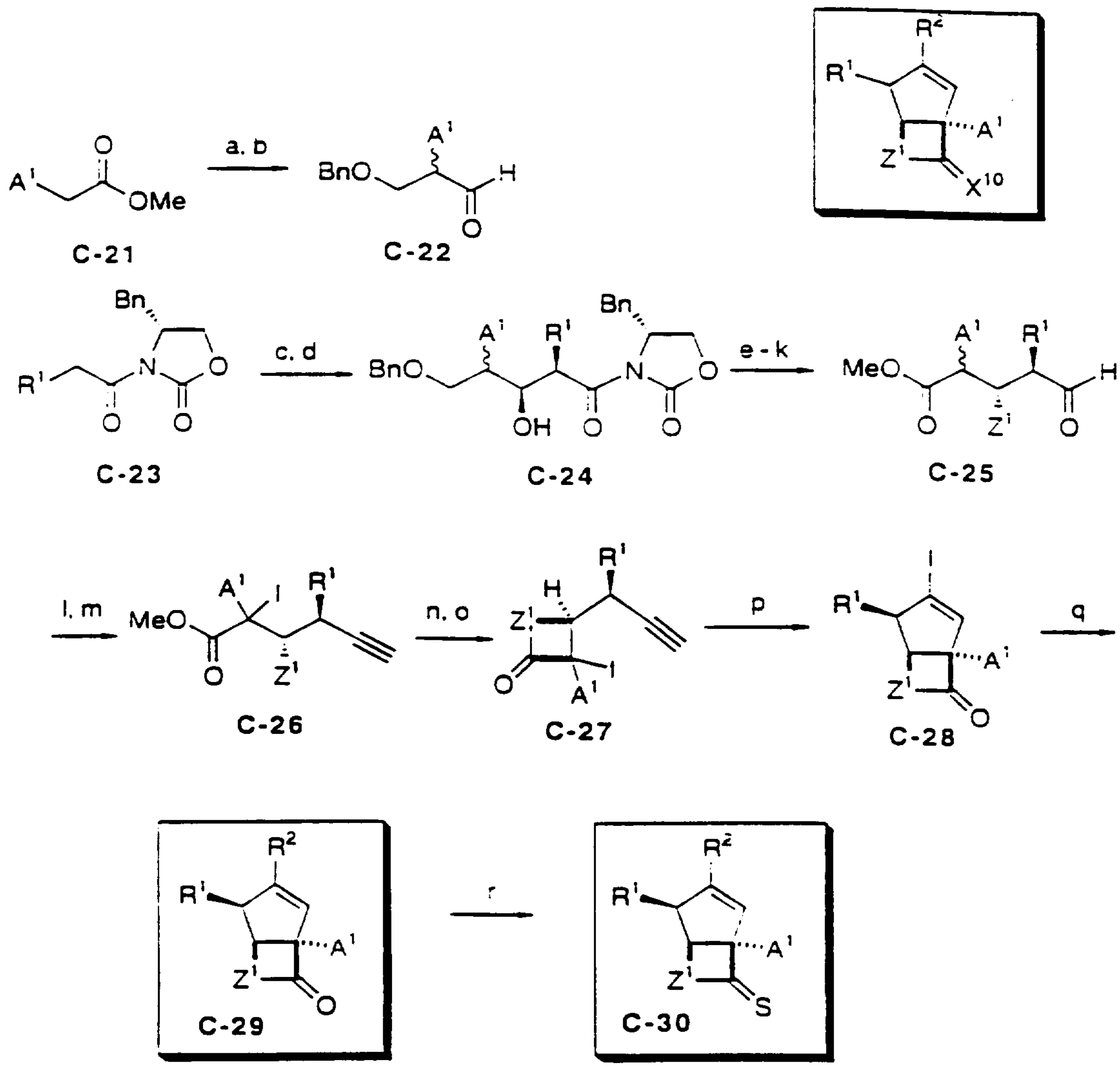
cyclizations. [Curran, D.P., *Comprehensive Organic Synthesis*, (1991) 4:779-831].

Esters **C-21** (Scheme 19), prepared by standard methods, are deprotonated and then alkylated with benzyloxybromo-methane and reduced with DIBAL-H to give aldehydes **C-22** as a racemic mixture. Addition of **C-22** to an boron enolate derived from chiral oxazolidinone imides **C-23** [Evans, D.A., et al., (1981)] yields **C-24** as a mixture of A^3 diastereomers. The configurational identity of the other two new stereocenters is established in this reaction and is not affected by the configuration of A^3.

Conversion to aldehydes **C-25** in a six-step procedure (for example, tosylation of the secondary hydroxyl, displacement with a nucleophile corresponding to Z^7, catalytic hydrogenolysis of the benzyl group, oxidation of the alcohol to the carboxylic acid and esterification) allows for preparation of the substrate for the intramolecular radical cycloaddition. Thus, treatment of the aldehyde with the Gilbert reagent [Gilbert, J.C., *et al.*, *J. Org. Chem.* (1982) 47:1837] and replacement of the enolizable hydrogen with iodine gives an acetylenes **C-26**, which, after deprotection of Z^7, if necessary, and cyclization with, for example, BOP-Cl, affords **C-27**.

Exposure of **C-27** to atom-transfer, intramolecular radical cyclization conditions [Curran, D.P., *et al.*, *Tet. Lett.* (1987) 28:2477 and Curran, D.P., *et al.*, *J. Org. Chem.* (1989) 54:3140] gives compounds **C-28**. This reaction deserves further comment. The stereochemistry of A^1 and the iodine atom are inconsequential because the radical generated from the iodine can interconvert under the reaction conditions. Further, only one of the radical diastereomers will be able to cyclize - that giving the *cis*-4,5 ring system depicted, because the *trans*-4,5 suffers from much greater ring strain.

Scheme 19



a) LDA; $BnOCH_2Br$ b) DIBAL-H c) Bu_2BOTf, Et_3N d) **C-22** e) TsCl, pyridine f) Nucleophile corresponding to Z^1 g) H_2, Pd/C h) Swern oxidation i) $NaClO_2$, NaH_2PO_4 j) CH_2N_2 k) $LiAlH_4$; Swern oxidation l) Base, Gilbert reagent m) LDA; TMS-Cl; I_2 n) LiOH, THF, water o) BOP-Cl p) $(Bu_3Sn)_2$, hv q) $(Ph_3P)_4Pd$, R^2-M r) Lawesson's reagent

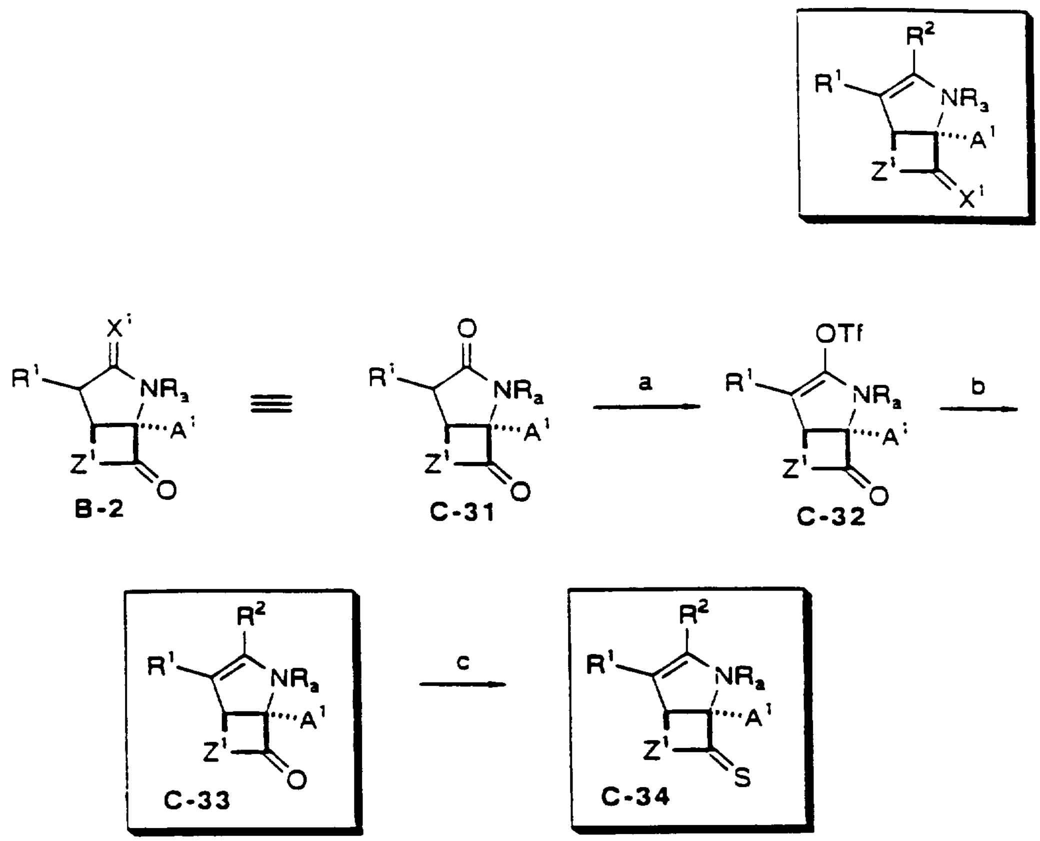
This cyclization is favored for two other reasons. First, it is a 5-endo-dig type cyclization and therefore favored according to Baldwin's rules for cyclization. [Baldwin, J. E., *J. Chem. Soc., Chem. Commun.* (1976) 734]. Second, the atom transfer conditions used are ideal because the resulting vinylic radical is very reactive and is rapidly quenched by the iodine radical. [Curran, D.P., *et al., J. Am. Chem. Soc.*, (1986) 108:2489].

Elaboration of **C-28** to **C-29** is accomplished using, for example, standard Stille [Stille, J.K., *Angew. Chem., Int. Ed. Engl.* (1986) 25:504] or Heck [Heck, R.F., *Comprehensive Organic Synthesis*, (1991) 4:833-863] type coupling conditions using a suitable metallic derivative (M is, e.g., tributylstannyl) of R^9. Sulfurization with Lawesson's reagent [Cava, M.P., et al., (1985)] gives compounds **C-30**, thus completing the preparation of all the compounds claimed in claims 8 and 39.

The preparation of the compounds covered by claims 9 and 40 relies primarily on intermediate **B-2**, whose preparation is described in Schemes 13a, 13b and 14 and discussed in the accompanying text. As shown in Scheme 20, **C-31** is deprotonated with a suitable base, and the resulting enolate is treated with, for example, the triflate source $PhN(Tf)_2$ [McMurry, J.E., *et al., Tet. Lett.* (1983) 24:979]. The vinyl triflates **C-32** are treated with, for example, a catalytic amount of $(Ph_3P)_4Pd$ and a suitable metallic derivative [Stille, J.K. (1986) and Heck, R.F. (1991)] (M is, e.g., tributylstannyl) of R^9, giving analogues **C-33**, which can be treated with Lawesson's reagent [Cava, M.P., et al., (1985)] gives compounds **C-34**, thus completing the preparation of all the compounds in this section.

The preparation of the compounds covered by claims 10 and 41 relies primarily on intermediate **A-38**, whose

Scheme 20



a) LiHMDS; $PhN(Tf)_2$ b) $(Ph_3P)_4Pd$, LiCl, R^2-M c) Lawesson's reagent

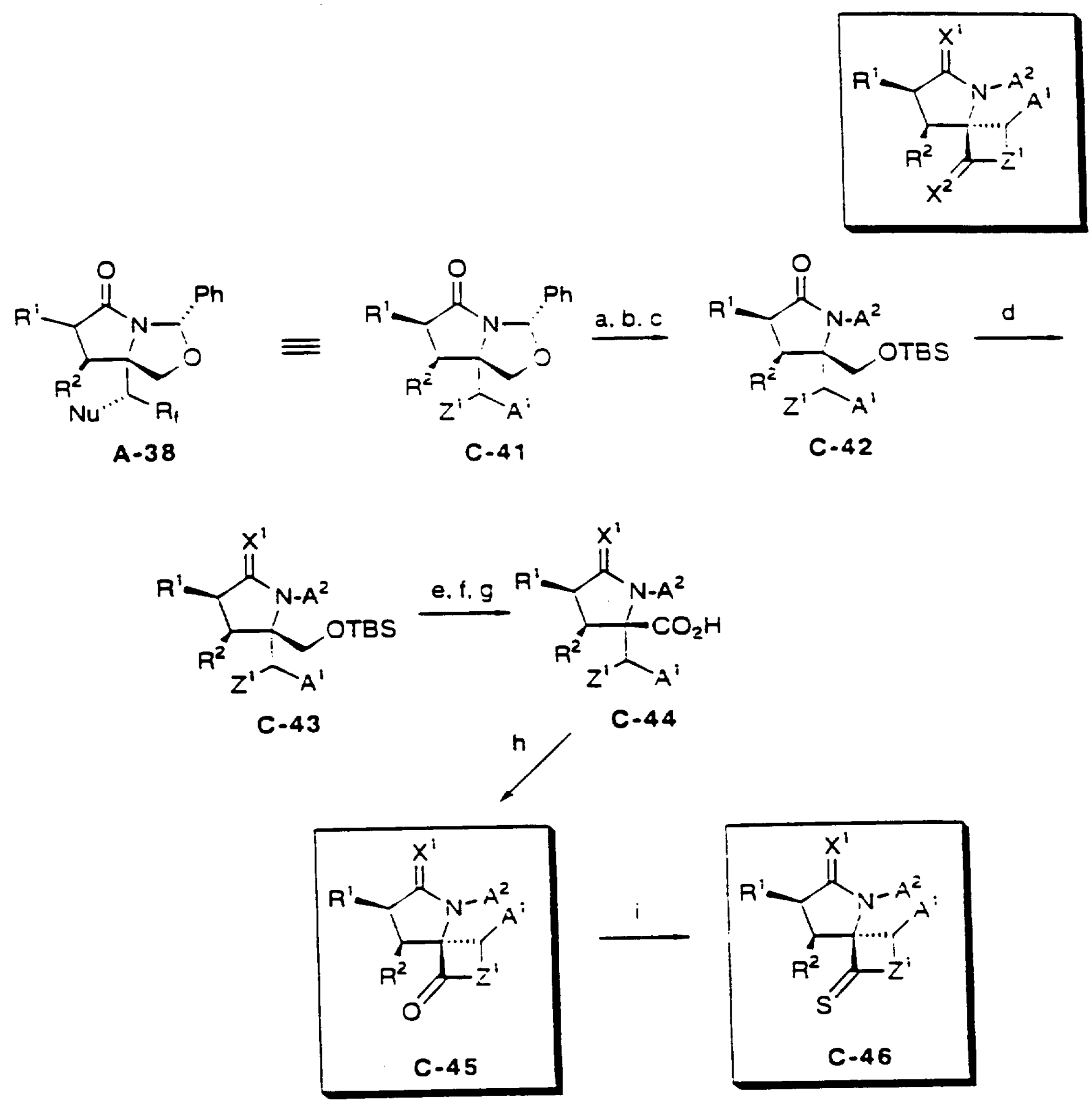
preparation is illustrated in Schemes 1, 5, and 6, and discussed in the accompanying text. Compounds of the type **C-41** (Scheme 21) can therefore be prepared with either the (*R*) or (*S*) configuration of Z^7 by following the procedures illustrated in Schemes 1-6, and discussed in the accompanying text.

Mild acidic hydrolysis of **C-41**, [Corey, E.J., et al., (1992a)] followed by protection of the primary hydroxyl [Corey, E.J., et al., (1972)] allows for installation of A^4 *via* standard alkylation procedures, [Challis, N. (1970)] giving **C-42**. Elaboration of these intermediates to **C-43** is performed as described for the analogous compounds in Scheme 8. Deprotection of the primary hydroxyl allows for oxidation to the carboxylic acids **C-44**, which are cyclized with, for example, BOP-Cl, giving analogues **C-45**, which can be treated with Lawesson's reagent [Cava, M.P., et al., (1985)] gives compounds **C-46**, thus completing the preparation of all the compounds in claims 10 and 41.

The preparation of the compounds covered by claims 11 and 42 follows the strategy illustrated in Schemes 1, 3, 4, 5, 8, and 9, and described in the accompanying text. Thus, as shown in Scheme 22, **C-51**, being analogous to **A-2** is elaborated to **C-52** following the same methods used in Scheme 1 using an aldehyde containing A^5 and a protected form of Z^7 in either the (*R*) or (*S*) configuration. Protection of the secondary hydroxyl gives compounds **C-52**, [Corey, E.J., et al., (1972)] which can be treated with a nucleophile corresponding to R^9 and subsequent quenching with acidic water, giving **C-53**. The reasons governing the indicated stereoselectivity of the nucleophilic addition and aqueous quenching have been discussed in the text accompanying Scheme 1.

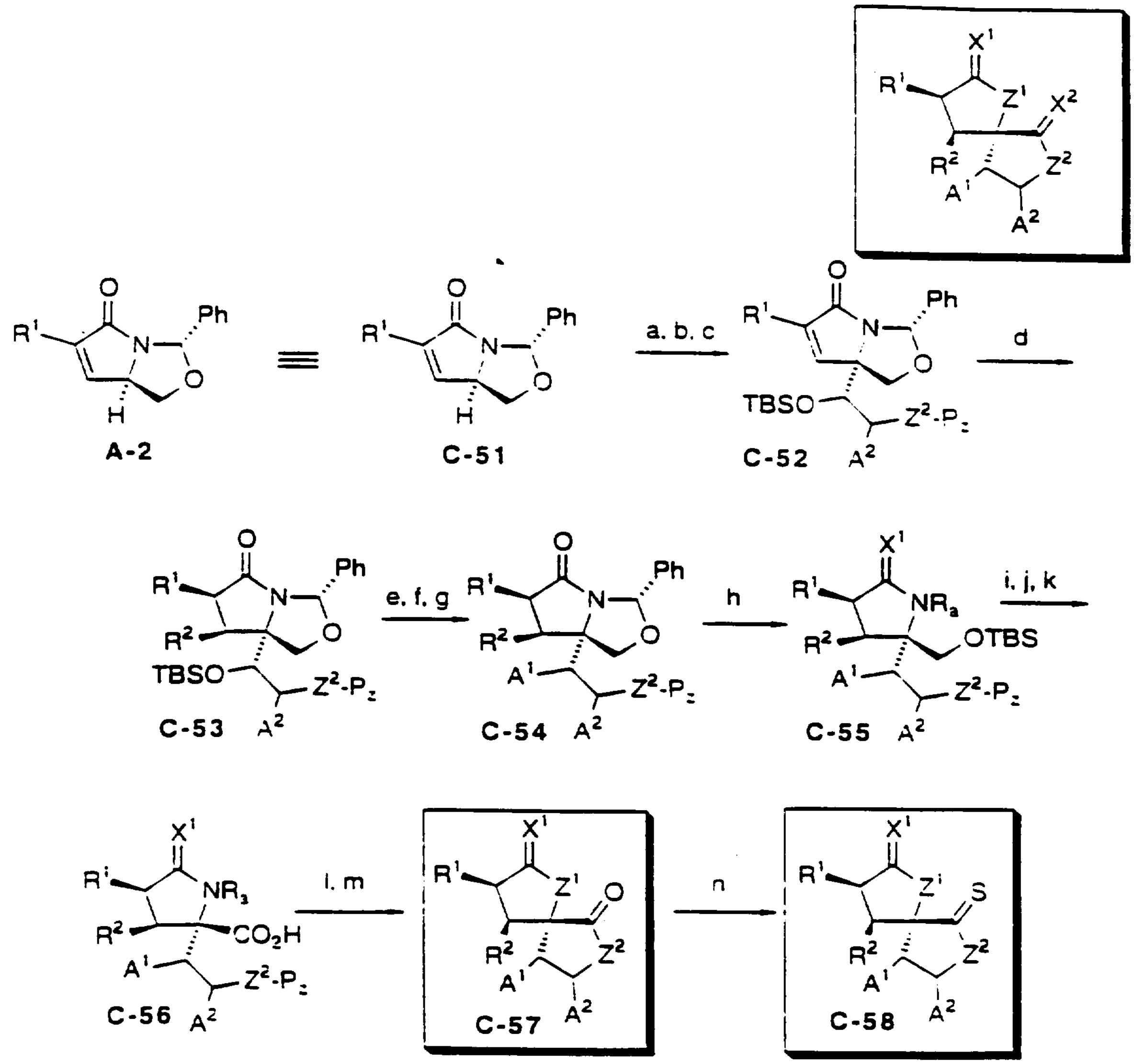
Deprotection and subsequent conversion of the secondary hydroxyl to the tosylate allows for

Scheme 21



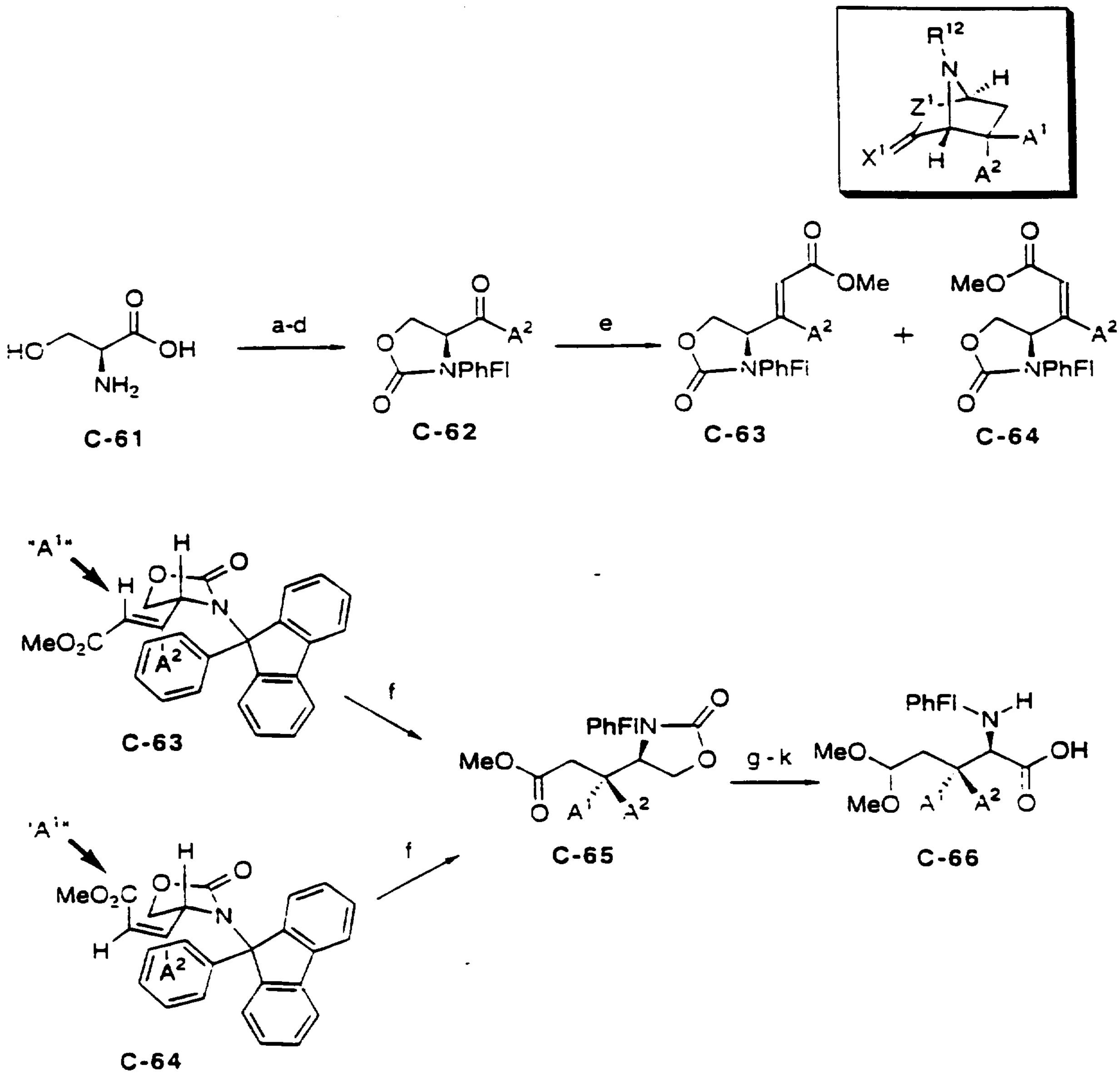
a) HCl, $HS(CH_2)_3SH$, CF_3CH_2OH b) TBS-Cl, imidazole, DMF c) base; electrophile corresponding to A^2 d) See Scheme 8 e) TBAF, THF f) Swern Oxidation g) $NaClO_2$, NaH_2PO_4 h) BOP-Cl i) Lawesson's reagent

Scheme 22



a) TBS-OTf, 2,6-lutidine b) $[(A^2)(Z^2\text{-}P_z)]CHCHO$, $SnCl_4$, Et_2O c) TBS-Cl, imidazole, DMF d) Nucleophile corresponding to R^2; acidic water e) TBAF, THF f) TsCl, pyridine g) Nucleophile corresponding to A^1 h) See Scheme 8 i) TBAF, THF j) Swern oxidation k) $NaClO_2$, NaH_2PO_4 l) deprotect Z^2, if necessary m) DCC n) Lawesson's reagent

Scheme 23



a-c) See Lubell, *et. al.* d) A^2MgBr. THF: acidic water e) $(MeO)_2\overset{\overset{O}{\|}}{P}CH_2CO_2Me$. NaH f) $(A^1)_2CuLi$ g) DIBAL-H h) MeOH. TsOH i) KOH. EtOH. reflux j) Swern oxidation k) $NaClO_2$, NaH_2PO_4

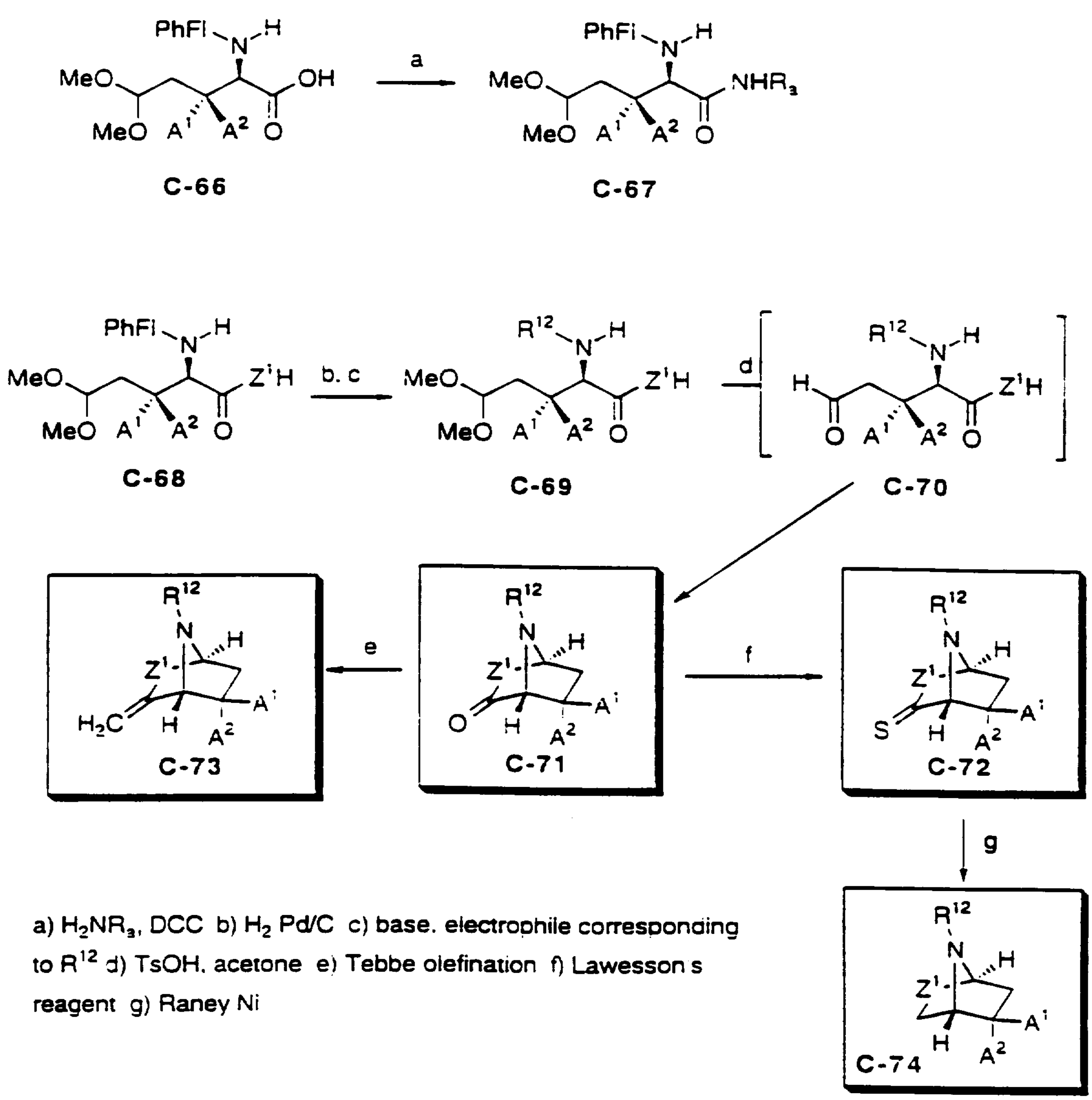
introduction of A^3 via displacement of the tosylate with a nucleophile corresponding to A^3 to give compounds of the type **C-54**. [Hanessian, S., (1980)]. Elaboration of these compounds to **C-55** is performed with the methods illustrated in Scheme 8 and described in the accompanying text. Deprotection of the primary hydroxyl allows for oxidation to the carboxylic acids **C-56**, which are cyclized with, for example, DCC, [Klausner, Y.S., et al., (1972)] following deprotection of Z^7, if necessary, to give analogues **C-57**. Sulfurization with Lawesson's reagent [Cava, M.P., (1985)] gives compounds **C-58**, thus completing the preparation of all the compounds in claims 12 and 42.

The preparation of the compounds covered by claims 13 and 43 relies primarily on the results of Lubell, et al., *J. Org. Chem.*, 1990 55:3511. Thus, as shown in Scheme 23, L-serine (**C-61**) is converted to ketones **C-62** by known procedures. (PhFl is an abbreviation of 9-phenyl-9-fluorenyl.) Standard Wadsworth-Emmons olefination of **C-62** yields either **C-63** or **C-64**, or a mixture thereof. The composition of the product mixture is of no consequence, since both olefin isomers yield the same diastereomer in the next reaction.

Thus, a cuprate reagent derived from A^1 approaches from the back face of both **C-63** or **C-64** since the very bulky PhFl group blocks the other face completely, giving **C-65** as the major diastereomer. **C-65** is converted to **C-66** in a five-step procedure (reduction of the methyl ester to the aldehyde and protection as the dimethyl acetal, deprotection of the oxazolidinone, and oxidation of the primary alcohol to the carboxylic acid.)

As depicted in Scheme 24, DCC-mediated coupling, for example, of **C-66** with an amine, yields amides **C-67**. Taken together, **C-66** and **C-67** comprise the general class of compounds **C-88**. The PhFl group is removed by

Scheme 24



a) H_2NR_3, DCC b) H_2 Pd/C c) base, electrophile corresponding to R^{12} d) TsOH, acetone e) Tebbe olefination f) Lawesson's reagent g) Raney Ni

catalytic hydrogenolysis, and the free amine alkylated with an electrophile corresponding to R^{12} (reductive amination with an aldehyde or ketone and $NaCNBH_3$ is another option) to give compounds **C-69**. Acidic hydrolysis of the dimethyl acetal yields intermediate aldehyde *C-70*, which cyclizes to analogues **C-73**.

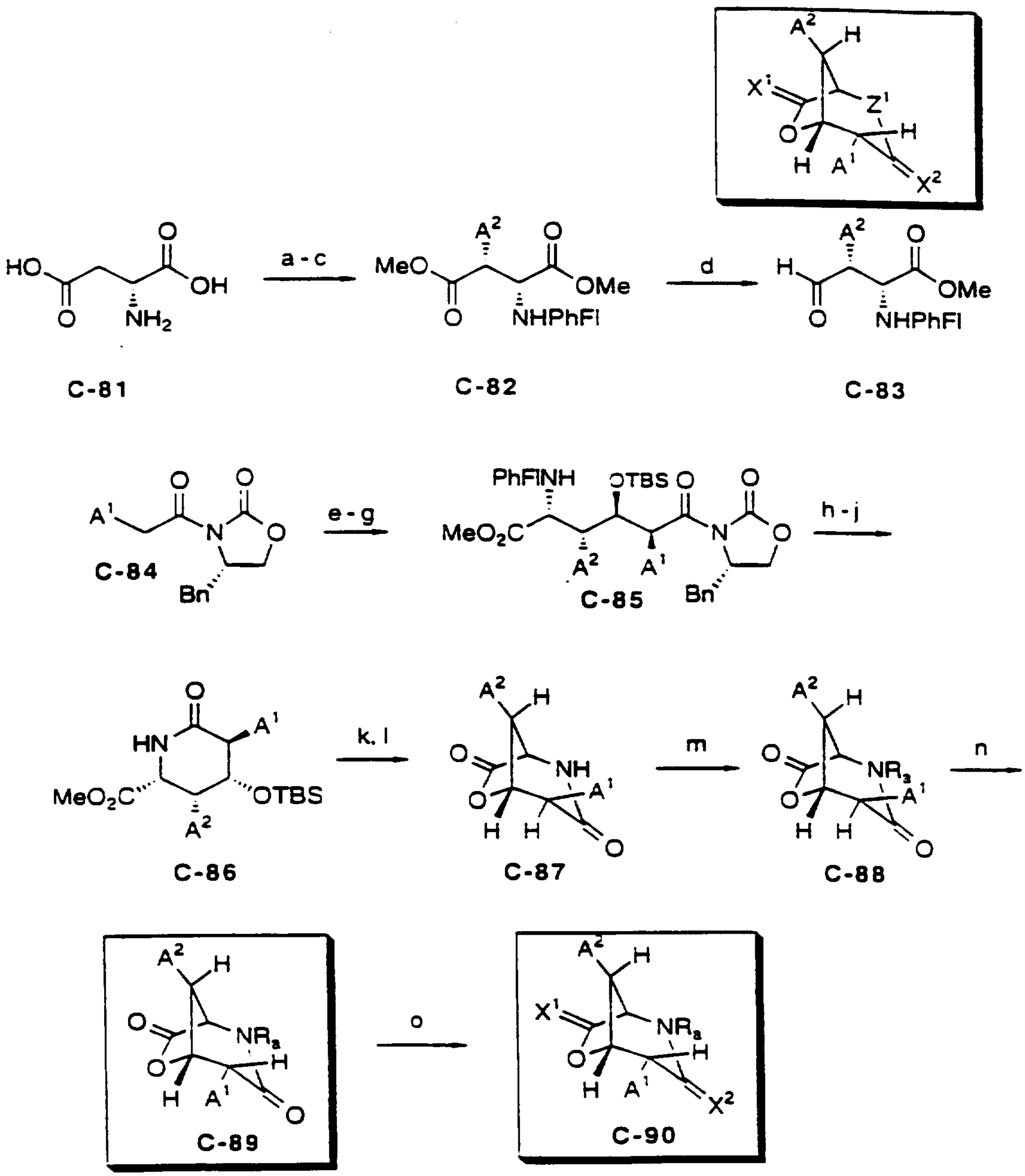
Tebbe olefination of **C-71** provides compounds **C-73**. Treatment of **C-71** with Lawesson's reagent provides compounds **C-72**, which can be desulfurized with Raney Nickel to give compounds **C-74**, thus completing the synthesis of all the compounds in claims 13 and 43.

The preparation of the compounds covered by claims 14 and 44 relies primarily on the results of Dener, et al., [*J. Org. Chem.*, 1993, 58:1159], and on those of Evans et al.,[*J. Am. Chem. Soc.*, 1981, 103:2127]. Thus, as shown in Scheme 25, D-aspartic acid (**C-81**) is protected and alkylated with an electrophile corresponding to A^6 to give compounds **C-82**, with the major diastereomer to be that shown. Dener et al.,[*J. Org. Chem.*, 1993, 58:1159]. (PhFl is an abbreviation of 9-phenyl-9-fluorenyl.) Site-specific DIBAL-H reduction (owing to the extreme size of the PhFl group) affords aldehyde **C-83**.

A diastereoselective boron-mediated aldol addition is the next step. Following the procedure of Evans, et al., *J. Am. Chem. Soc.*, (1981) 103:2127, one obtains **C-85**, after TBS protection of the secondary hydroxyl. The oxazolidinone is removed with lithium hydroperoxide, the PhFl group removed with catalytic hydrogenolysis, and lactam formation accomplished under, for example, DCC conditions, to give **C-86**. Removal of the TBS group with TBAF and lactone formation (saponification of the methyl ester with LiOH, followed by DCC coupling may be necessary) yields **C-87**, which is elaborated to **C-88** by alkylating the amide nitrogen with a nucleophile

corresponding to R_d. Epimerization with DBU provides access to compounds **C-89**. Treatment of C-89 with Lawesson's reagent, Cava et al., *Tetrahedron*, 1985, 41: 5061, yields compounds C-90, thus completing the preparation of all compounds in this section. Examples 13-21 below are individual syntheses.

Scheme 25



a-c) See Dener, *et. al.* d) DIBAL-H e) Bu_2OTf f) **C-83** g) TBS-Cl, imidazole, DMF h) LiOOH, THF, H_2O i) H_2, Pd/C j) DCC k) TBAF, THF l) cyclize m) base, electrophile corresponding to R_3 n) DBU o) Lawesson's reagent

Use

The disclosed compounds are used to treat conditions mediated directly by the proteolytic function of the proteasome such as muscle wasting, or mediated indirectly via proteins which are processed by the proteasome such as NF-κB. The proteasome participates in the rapid elimination and post-translational processing of proteins (e.g., enzymes) involved in cellular regulation (e.g., cell cycle, gene transcription, and metabolic pathways), intercellular communication, and the immune response (e.g., antigen presentation). Specific examples discussed below include β-amyloid protein and regulatory proteins such as cyclins and transcription factor NF-κB. Treating as used herein includes reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in manner to improve or stabilize the subject's condition.

Alzheimer's disease is characterized by extracellular deposits of β-amyloid protein (β-AP) in senile plaques and cerebral vessels. β-AP is a peptide fragment of 39 to 42 amino acids derived from an amyloid protein precursor (APP). At least three isoforms of APP are known (695, 751, and 770 amino acids). Alternative splicing of mRNA generates the isoforms; normal processing affects a portion of the β-AP sequence, thereby preventing the generation of β-AP. It is believed that abnormal protein processing by the proteasome contributes to the abundance of β-AP in the Alzheimer brain. The APP-processing enzyme in rats contains about ten different subunits (22 kDa - 32 kDa). The 25 kDa subunit has an N-terminal sequence of X-Gln-Asn-Pro-Met-X-Thr-Gly-Thr-Ser, which is identical to the β-subunit of human macropain. Kojima, S. et al., *Fed. Eur. Biochem. Soc.*, (1992) 304:57-60. The APP-processing enzyme cleaves at the Gln^{15}-Lys^{16} bond; in the presence of

calcium ion, the enzyme also cleaves at the Met^{-1}-Asp^{1} bond, and the Asp^{1}-Ala^{2} bonds to release the extracellular domain of β-AP.

One embodiment, therefore, is a method of treating Alzheimer's disease, including administering to a subject an effective amount of a compound (e.g., pharmaceutical composition) having a formula disclosed herein. Such treatment includes reducing the rate of β-AP processing, reducing the rate of β-AP plaque formation, and reducing the rate of β-AP generation, and reducing the clinical signs of Alzheimer's disease.

Other embodiments of the invention relate to cachexia and muscle-wasting diseases. The proteasome degrades many proteins in maturing reticulocytes and growing fibroblasts. In cells deprived of insulin or serum, the rate of proteolysis nearly doubles. Inhibiting the proteasome reduces proteolysis, thereby reducing both muscle protein loss and the nitrogenous load on kidneys or liver. Proteasome inhibitors are useful for treating conditions such as cancer, chronic infectious diseases, fever, muscle disuse (atrophy) and denervation, nerve injury, fasting, renal failure associated with acidosis, and hepatic failure. See, e.g., Goldberg, U.S. Pat. No. 5,340,736 (1994). Embodiments of the invention therefore encompass methods for: reducing the rate of muscle protein degradation in a cell, reducing the rate of intracellular protein degradation, reducing the rate of degradation of p53 protein in a cell, and inhibiting the growth of p53-related cancers). Each of these methods includes the step of contacting a cell (in vivo or in vitro, e.g., a muscle in a subject) with an effective amount of a compound (e.g., pharmaceutical composition) of a formula disclosed herein.

Another protein processed by the proteasome is NF-κB, a member of the Rel protein family. The Rel family of transcriptional activator proteins can be divided into two groups. The first group requires proteolytic processing, and includes p50 (NF-κB1, 105 kDa) and p52 (NF-κ2, 100 kDa). The second group does not require proteolytic processing, and includes p65 (RelA, Rel (c-Rel), and RelB). Both homo- and heterodimers can be formed by Rel family members; NF-κB, for example, is a p50-p65 heterodimer. After phosphorylation and ubiquitination of IκB and p105, the two proteins are degraded and processed, respectively, to produce active NF-κB which translocates from the cytoplasm to the nucleus. Ubiquitinated p105 is also processed by purified proteasomes. Palombella et al., *Cell* (1994) 78:773-785. Active NF-κB forms a stereospecific enhancer complex with other transcriptional activators and, e.g., HMG I(Y), inducing selective expression of a particular gene.

NF-κB regulates genes involved in the immune and inflammatory response, and mitotic events. For example, NF-κB is required for the expression of the immunoglobulin light chain κ gene, the IL-2 receptor α-chain gene, the class I major histocompatibility complex gene, and a number of cytokine genes encoding, for example, IL-2, IL-6, granulocyte colony-stimulating factor, and IFN-β. Palombella et al., (1994). Some embodiments of the invention include methods of affecting the level of expression of IL-2, MHC-I, IL-6, IFN-β or any of the other previously-mentioned proteins, each method including administering to a subject an effective amount of a compound of a formula disclosed herein.

NF-κB also participates in the expression of the cell adhesion genes that encode E-selectin, P-selectin, ICAm, and VCAM-1, Collins, T., *Lab. Invest.* (1993)

68:499-508. One embodiment of the invention is a method for inhibiting cell adhesion (e.g., cell adhesion mediated by E-selectin, P-selectin, ICAm, or VCAM-1), including contacting a cell with (or administering to a subject) an effective amount of a compound (e.g., pharmaceutical composition) having a formula disclosed herein.

NF-κB also binds specifically to the HIV-enhancer/promoter. When compared to the Nef of mac239, the HIV regulatory protein Nef of pbj14 differs by two amino acids in the region which controls protein kinase binding. It is believed that the protein kinase signals the phosphorylation of I-κB, triggering IκB degradation through the ubiquitin-proteasome pathway. After degradation, NF-κB is released into the nucleus, thus enhancing the transcription of HIV. Cohen, J., *Science*, (1995) 267:960. Two embodiments of the invention are a method for inhibiting or reducing HIV infection in a subject, and a method for decreasing the level of viral gene expression, each method including administering to the subject an effective amount of a compound of a formula disclosed herein.

Complexes including p50 are rapid mediators of acute inflammatory and immune responses. Thanos, D. and Maniatis, T., *Cell* (1995) 80:529-532. Intracellular proteolysis generates small peptides for presentation to T-lymphocytes to induce MHC class I-mediated immune responses. The immune system screens for autologous cells that are virally infected or have undergone oncogenic transformation. Two embodiments of the invention are a method for inhibiting antigen presentation in a cell, including exposing the cell to a compound of a formula described herein, and a method for suppressing the immune system of a subject (e.g., inhibiting transplant rejection), including administering

to the subject an effective amount of a compound of a formula described herein.

In addition, the invention provides a method for treating inflammation, wherein the method includes administering to a subject an effective anti-inflammatory amount of a pharmaceutical composition containing a compound of a formula described herein. Inflammation can be a primary or secondary responses associated with (a) injury such as a cut, laceration, puncture wound, (b) infection (including infected surgical incisions) by one or more viruses, bacteria, mycobacteria, microorganisms, parasites, and fungi, (c) allergies, (d) a disease state, (e) surgery (e.g., transplantation), or (f) a combination thereof.

Allergies are primary inflammatory responses to antigens or allergens. Sources of allergens include plants (e.g., grass or tree pollen), animals (e.g., dander, venom, urine, execreta from dogs, cats, insects, and snakes), and fungi. In addition to allergens such as rye grass, ragweed, and Japanese cedar pollen, certain foods or food components (e.g., eggs, milk, shellfish, strawberries, chocolate), vaccines, and drugs (e.g., penicillin) can induce allergic reactions in certain individuals.

Disease states include rheumatoid arthritis, scleroderma, rheumatic fever, inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), diabetes mellitus, myasthenia gravis, multiple sclerosis, Guillain-Barre syndrome, conjuctiva of the eye, systemic lupus erythematosus, encephalitis, Adult Respiratory Distress Syndrome, psoriasis, emphysema, Alzheimer's disease, and muscular dystrophy.

The invention provides a method of treating inflammation induced by organ or tissue transplantation. This method includes administering to a patient who has

undergone or is about to undergo transplantation a composition containing a compound having a formula disclosed herein. Transplantations include bone marrow, solid organ (e.g., kidney, lungs, heart, pancreas, liver, and skin), or tissues.

Certain proteasome inhibitors block both degradation and processing of ubquitinated NF-κB in vitro and in vivo. Proteasome inhibitors also block IκB-α degradation and NF-κB activation, Palombella, *et al.*; and Traenckner, *et al.*, *EMBO J.* (1994) 13:5433-5441. One embodiment of the invention is a method for inhibiting IκB-α degradation, including contacting the cell with a compound of a formula described herein. A further embodiment is a method for reducing the cellular content of NF-κB in a cell, muscle, organ, or subject, including contacting the cell, muscle, organ, or subject with a compound of a formula described herein.

Other eukaryotic transcription factors that require proteolytic processing include the general transcription factor TFIIA, herpes simplex virus VP16 accessory protein (host cell factor), virus-inducible IFN regulatory factor 2 protein, and the membrane-bound sterol regulatory element-binding protein 1.

Other embodiments of the invention are methods for affecting cyclin-dependent eukaryotic cell cycles, including exposing a cell (in vitro or in vivo) to a compound of a formula disclosed herein. Cyclins are proteins involved in cell cycle control. The proteasome participates in the degradation of cyclins. Examples of cyclins include mitotic cyclins, G1 cyclins, (cyclin B). Degradation of cyclins enables a cell to exit one cell cycle stage (e.g., mitosis) and enter another (e.g., division). It is believed all cyclins are associated with $p34^{cdc2}$ protein kinase or related kinases. The proteolysis targeting signal is localized to amino acids

42-RAALGNISEN-50 (destruction box). There is evidence that cyclin is converted to a form vulnerable to a ubiquitin ligase or that a cyclin-specific ligase is activated during mitosis. Ciechanover, A., *Cell*, (1994) 79:13-21. Inhibition of the proteasome inhibits cyclin degradation, and therefore inhibits cell proliferation (e.g., cyclin-related cancers). Kumatori et al., *Proc. Natl. Acad. Sci. USA* (1990) 87:7071-7075. One embodiment of the invention is a method for treating a proliferative disease in a subject (e.g., cancer, psoriasis, or restenosis), including administering to the subject an effective amount of a compound of a formula disclosed herein. Chronic or acute inflammation can result from transplantation rejection, arthritis, rheumatoid arthritis, infection, dermatosis, inflammatory bowel disease, asthma, osteoporosis, and autoimmune diseases. Rejection or inflammation can occur in transplanted tissues or organs of any type, including heart, lung, kidney, liver, skin grafts, and tissue grafts. The invention also encompasses a method for treating cyclin-related inflammation in a subject, including adminstering to a subject an effective amount of a compound of a formula described herein.

Additional embodiments are methods for affecting the proteasome-dependent regulation of oncoproteins and methods of treating or inhibiting cancer growth, each method including exposing a cell (*in vivo*, e.g., in a subject or *in vitro*) to a compound of a formula disclosed herein. HPV-16 and HPV-18-derived E6 proteins stimulate ATP- and ubiquitin-dependent conjugation and degradation of p53 in crude reticulocyte lysates. The recessive oncogene p53 has been shown to accumulate at the nonpermissive temperature in a cell line with a mutated thermolabile E1. Elevated levels of p53 may lead to apoptosis. Examples of proto-oncoproteins degraded by

the ubiquitin system include c-Mos, c-Fos, and c-Jun. One embodiment is a method for treating p53-related apoptosis, including administering to a subject an effective amount of a compound of a formula disclosed herein.

Treatment of cancer prevents, alleviates, or ameliorates one or more primary or secondary phenomena associated with the initiation, progression, and metastasis of tumors, especially malignant tumors, e.g., a growth of tissue wherein cell multiplication is uncontrolled. Malignant tumors show a greater degree of anaplasia than do benign tumors. The invention provides a method of treating cancer including administering to a subject an effective anti-cancer amount of a pharmaceutical composition described herein, wherein the cancer is selected from carcinoma, lymphoma, sarcoma, and myeloma.

Examples of carcinomas include adenocarcinoma, acinic cell adenocarcinoma, adrenal cortical carcinomas, alveoli cell carcinoma, anaplastic carcinoma, basaloid carcinoma, basal cell carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, renaladinol carcinoma, embryonal carcinoma, anometroid carcinoma, fibrolamolar liver cell carcinoma, follicular carcinomas, giant cell carcinomas, hepatocellular carcinoma, intraepidermal carcinoma, intraepithelial carcinoma, leptomanigio carcinoma, medullary carcinoma, melanotic carcinoma, menigual carcinoma, mesometonephric carcinoma, oat cell carcinoma, squamal cell carcinoma, sweat gland carcinoma, transitional cell carcinoma, and tubular cell carcinoma. Examples of sarcoma include amelioblastic sarcoma, angiolithic sarcoma, botryoid sarcoma, endometrial stroma sarcoma, ewing sarcoma, fascicular sarcoma, giant cell sarcoma, granulocytic sarcoma, immunoblastic sarcoma, juxaccordial osteogenic sarcoma, coppices sarcoma,

leukocytic sarcoma (also known as leukemia), lymphatic sarcoma (also known as lympho sarcoma), medullary sarcoma, myeloid sarcoma (also known as granulocytic sarcoma), austiogenci sarcoma, periosteal sarcoma, reticulum cell sarcoma (also known as histiocytic lymphoma), round cell sarcoma, spindle cell sarcoma, synovial sarcoma, and telangiectatic audiogenic sarcoma. Examples of lymphomas include Hodgkin's disease and lymphocytic lymphomas, such as Burkitt's, nodular poorly-differentiated lymphocytic (NPDL), nodular mixed lymphocytic (NML), NH (nodular histiocytic), and diffuse lymphomas. Additional carcinomas include neural blastoma, glioblastoma, astrocytoma, melanoma, leiomyo sarcoma, multiple myeloma, and Hemangioma.

A tripeptide aldehyde protease inhibitor (benzyloxycarbonyl (Z)-Leu-Leu-leucinal induces neurite outgrowth in PC12 cells at an optimal concentration of 30 nM, Tsubuki et al., *Biochem. and Biophys. Res. Comm.* (1993) 196:1195-1201. Peptide aldehydes have been shown to inhibit the chymotryptic activity of the proteasome. Vinitsky, et al., 1992, Tsubuki et al., 1993. One embodiment of the invention is a method of promoting neurite outgrowth, including administering to the subject a compound of a formula disclosed herein.

Finally, the disclosed compounds are also useful as diagnostic agents (e.g., in diagnostic kits or for use in clinical laboratories) for screening for proteins (e.g., enzymes, transcription factors) processed by the proteasome. The disclosed compounds are also useful as research reagents for specifically binding the X/MB1 subunit or α-chain and inhibiting the proteolytic activities associated with it. For example, the activity of (and specific inhibitors of) other subunits of the proteasome can be determined.

Most cellular proteins are subject to proteolytic processing during maturation or activation. Lactacystin can be used to determine whether a cellular, developmental, or physiological process or output is regulated by the proteolytic activity of the proteasome. One such method includes obtaining an organism, an intact cell preparation, or a cell extract; exposing the organism, cell preparation, or cell extract to a compound of a formula disclosed herein; exposing the compound-exposed organism, cell preparation, or cell extract to a signal, and monitoring the process or output. The high selectivity of the compounds disclosed herein permits rapid and accurate elimination or implication of the proteasome in a given cellular, developmental, or physiological process.

The compounds and compositions of the invention are useful in several methods including a method of treating inflammation, comprising administering to a subject an effective anti-inflammatory amount of a pharmaceutical composition described herein (e.g., a lactacystin thioester, a lactone, or a lactam); wherein the inflammation is associated with injury or infection; wherein the inflammation is associated with an allergy or asthma; wherein the inflammation is associated with a disease state selected from rheumatoid arthritis, scleroderma, rheumatic fever, inflammatory bowel disease, diabetes mellitus, myasthenia gravis, multiple sclerosis, Guillan-Barre syndrome, conjunctiva of the eye, systemic lupus erythematosus, encephalitis, Adult Respiratory Distress Syndrome, emphysema, Alzheimer's disease, and muscular dystrophy; wherein the inflammation is associated with transplantation of bone marrow or a solid organ selected from kidney, lung, heart, pancreas, liver, and skin, and the composition is administered before, during, or after transplantation; wherein the

pharmaceutical composition is administered orally; or combinations thereof.

The invention also provides a method of treating cancer, comprising administering to a subject an effective anti-cancer amount of a pharmaceutical composition described herein; a method for treating psoriasis, comprising administering to a subject an effective amount of a pharmaceutical composition described herein; and a method for treating restenosis, comprising administering to a subject an effective amount of a pharmaceutical composition described herein. Preferred compounds and compositions include various lactacystin thioesters, and lactacystin β-lactone analogs, including the β-lactone itself.

Formulation and Administration

The methods of the invention contemplate treatment of animal subjects, such as mammals (e.g., higher primates, and especially humans). The invention encompasses pharmaceutical compositions which include novel compounds described herein, and pharmaceutical compositions which include compounds described and first recognized herein as proteasome inhibitors, such as lactacystin.

Pharmaceutically acceptable salts may be formed, for example, with 1, 2, 3, or more equivalents of hydrogen chloride, hydrogen bromide, trifluoroacetic acid, and others known to those in the art of drug formulation. Compounds of the invention can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable non-toxic excipients and carriers. A pharmaceutical composition of the invention may contain more than one compound of the invention, and/or may also contain other therapeutic compounds not encompassed by the invention, such as anti-inflammatory, anti-cancer, or other agents. A subject may have more

than one type of inflammation, or more than one type of cancer, a combination of allergies, or a mixture of the above conditions for which the disclosed compounds are useful. A compound of the invention may be administered in unit dosage form, and may be prepared by any of the methods well known in the pharmaceutical art, for example, as described in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., Easton, PA, 1980). The invention also encompasses a packaged drug, containing a pharmaceutical composition formulated into individual dosages and printed instructions for self-administration.

Compounds disclosed herein as proteasome inhibitors may be prepared for use in parenteral administration in the form of solutions or liquid suspensions; for oral administration (preferable), particularly in the form of tablets or capsules; or intranasally, particularly in the form of powders, gels, oily solutions, nasal drops, aerosols, or mists. Formulations for parenteral administration may contain as common excipients sterile water or sterile saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Controlled release of a compound of the invention may be obtained, in part, by use of biocompatible, biodegradable polymers of lactide, and copolymers of lactide/glycolide or polyoxyethylene/ polyoxypropylene. Additional parental delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain lactose, polyoxyethylene-9-lauryl ether, glycocholate, or deoxycholate. Formulations for buccal administration may include glycocholate; formulations for vaginal administration may include citric acid.

The concentration of a disclosed compound in a pharmaceutically acceptable mixture will vary depending on several factors, including the dosage of the compound to be administered, the pharmacokinetic characteristics of the compound(s) employed, and the route of administration. In general, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.1-10% w/v of compound for parenteral administration. Typical dose ranges are from about 0.1 to about 50 mg/kg of body weight per day, given in 1-4 divided doses. Each divided dose may contain the same or different compounds of the invention. The dosage will be an effective amount depending on several factors including the overall health of a patient, and the formulation and route of administration of the selected compound(s).

The effective amount of the active compound used to practice the present invention for treatment of conditions directly or indirectly mediated by the proteasome varies depending upon the manner of administration, the age and the body weight of the subject and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such amount of the active compound as determined by the attending physician or veterinarian is referred to herein as "effective amount".

Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific examples are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are hereby incorporated by reference.

EXAMPLES

Example 1

Synthesis of [^{3}H] lactacystin

Lactacystin was prepared from the β-lactone, which was tritiated by an oxidation-reduction sequence. Unlabeled β-lactone was oxidized at C9 to the ketone with the Dess-Martin periodinane in dichloromethane and then reduced with [^{3}H]$NaBH_4$ (NEN, 13.5 Ci/mmol) in 1,2-dimethoxyethane/1% H_2O to afford tritiated β-lactone along with its C9-epimer (2:3 ratio). The isomeric β-lactones were separated by HPLC (Rainin Microsorb SiO_2 column, 4.6 x 100 mm, 10% *i*-Pr-OH in hexane) and reacted separately with *N*-acetylcysteine (0.5 M) and Et_3N (1.5 M) in CH_3CN to afford the lactacystin (9*S*) and its C9-epimer (9*R*), which were purified from their respective reaction mixtures by reverse-phase HPLC (TSK ODS-80T_m column, 4.6 x 250 mm, 10% CH_3CN/0.1% CF_3CO_2H in H_2O).

Example 2

Fluorography of crude cell and tissue extracts

Crude Neuro 2A extracts (~11 μg total protein/lane) and crude bovine brain extracts (~54 μg total protein/lane) were treated as follows: (1) 10 μM [^{3}H]lactacystin; (2) 10 μM [^{3}H]lactacystin and 1 mM cold lactacystin; (3) 10 μM [^{3}H]lactacystin; (4) 10 μM [^{3}H]lactacystin and 1 mM cold lactacystin; (5) 10 μM [^{3}H]lactacystin and 1 mM cold β-lactone; (6) 10 μM [^{3}H]lactacystin and 1 mM cold phenylacetyl lactacystin; (7) 10 μM [^{3}H]lactacystin and 1 mM cold lactacystin amide; (8) 10 μM [^{3}H]lactacystin and 1 mM cold dihydroxy acid; (9) 10 μM [^{3}H]lactacystin and 1 mM cold 6-deoxylactacystin; (10) 10 μM [^{3}H]lactacystin and 1 mM cold (6*R*,7*S*)-lactacystin (6-*epi*, 7-*epi*); (11) 10 μM [^{3}H]lactacystin and 1 mM cold des(hydroxyisobutyl)-lactacystin. Crude extracts from Neuro2A neuroblastoma

cells or bovine brain were incubated in the presence of 10 μM [^3H]lactacystin in the presence or absence of 1 mM cold competitor (added simultaneously) for 24 h at room temperature, followed by SDS-polyacrylamide gel electrophoresis (0.75-mm-thick 12% polyacrylamide gel). The gel was stained in 0.1% Coomassie brilliant blue R-250, 12% acetic acid, 50% MeOH, and then destained in 12% acetic acid, 50% MeOH, followed by impregnation with EN^{3}HANCE (NEN) and precipitation of the fluor with water. The gel was dried on Whatman filter paper in a gel dryer at 65°C for 30 min and then exposed to Kodak SB film at -78°C.

Example 3

Separation of purified protein complex (20S proteasome)

Bovine brain was frozen in liquid N_2. A total of two kg of brain was dry homogenized in a Waring blender for one minute. All operations were performed at 4°C, except as noted. The following buffer (6 liters total) was then added (pH 7.7): 18.25 mM K_2HPO_4, 6.75 mM KH_2PO_4, 0.27 M sucrose, 2 mM EDTA, 2 mM EGTA, 25 mM NaF, 5 mM tetrasodium pyrophosphate, 5 μg/ml each of leupeptin and pepstatin A, and 5 mM β-mercaptoethanol. The tissue was wet homogenized in the Waring blender for two minutes. The homogenate was centrifuged at 5,000 g for 15 min and then at 12,000 g for 30 min. Ammonium sulfate was added to the supernatant to 50% saturation, and the sample spun at 10,000 g for 20 min. The 50-60% saturation ammonium sulfate fraction, containing the lactacystin-binding activity as determined by SDS-PAGE and fluorography of samples incubated with radioactive compound, was then dialyzed overnight against 20 mM MES-NaOH, pH 5.6, 5 mM β-mercaptoethanol, followed by SP-sepharose chromatography (Pharmacia SP-sepharose, fast flow; 120-ml bed volume column) with 20 mM MES-NaOH, pH 5.6, 5 mM

β-mercaptoethanol and an NaCl gradient from 0-0.3 M (500-ml gradient; flow rate = 2 ml/min).

After pooling and diluting the relevant fractions, the pH was adjusted to 8 with 1 M Tris-HCl, pH 8. Q-sepharose chromatography (Pharmacia Q-sepharose, fast flow; 16-ml bed volume column) was performed with 20 mM Tris-HCl, pH 8, 5 mM β-mercaptoethanol and a NaCl gradient from 0-0.5 M (120-ml gradient; flow rate = 1 ml/min). The relevant fractions were pooled, concentrated, and then applied to a Pharmacia Superose 6 gel filtration column (10 mM Tris-HCl, 1 mM EDTA, pH 8, 5 mM β-mercaptoethanol; flow rate = 0.5 ml/min), with the lactacystin-binding activity corresponding to a single high molecular weight peak. This peak was isolated and treated with 10 μM [^{3}H]lactacystin or 10 μM [^{3}H]β-lactone for 24 h at 25°C. Trifluoroacetic acid (TFA) was added to 0.1%, and the sample was allowed to stand at room temperature for 20 min. Reverse-phase HPLC was then carried out at room temperature using a Vydac C4 column (300 Å/4.6 x 150 mm) with 20-40% acetonitrile/0.1% TFA over 10 min and then 40-55% acetonitrile/0.1% TFA over 30 min (flow rate = 0.8 ml/min). An IN/US β-RAM in-line scintillation detector was used to monitor radioactivity.

The lactacystin-binding proteins were purified from bovine brain, and both were found to reside in the same high-molecular weight protein complex by gel-filtration chromatography. Treatment of the complex with [^{3}H]lactacystin did not cause its dissociation, and the radioactivity uniquely comigrated with the complex. The molecular weight of the complex was estimated to be 700 kDa, and SDS-PAGE revealed that it consisted of numerous proteins with molecular weights of ~24-32 kDa. Edman degradation of blotted protein revealed the sequences of several proteasome subunits in the 24 kDa band, leading to a tentative identification of the

complex as the 20S proteasome. After the complex was treated with [^{3}H]lactacystin and subjected to reverse-phase HPLC to separate the proteasome subunits, eleven to twelve distinct peaks were resolved. However, the radioactivity was associated exclusively with the first two peaks, and predominantly with the second. These first two peaks were judged to be homogeneous by Coomassie blue staining of SDS-polyacrylamide gels and by sequencing of tryptic fragments, while some of the subsequently eluting, larger peaks were clearly not homogeneous. The ratio of counts incorporated into the first peak versus the second peak varied with the batch of protein, the length of incubation, and the ligand. The first peak is labeled more slowly, and the degree of labeling of the first peak relative to the second is greater with [^{3}H]lactacystin than with the [^{3}H]β-lactone at any given time. A one or two hour reaction with [^{3}H]β-lactone results in only trace labeling of the first peak, while a 24 hour reaction with [^{3}H]β-lactone or [^{3}H]lactacystin results in significant labeling of this peak. The selectivity runs opposite to the relative chemical reactivity of the two compounds, and this finding suggests that the *N*-acetylcysteine moiety of lactacystin may facilitate recognition by this secondary protein. The first peak to elute from the reverse-phase HPLC column contained only a ~32 kDa protein, which corresponded to the 32 kDa secondary lactacystin-binding protein identified earlier.

Example 4

Amino acid sequence of purified bovine lactacystin-binding proteins

Purified 20S proteasome (purified as described in Example 3) was incubated for 24 h at room temperature with 10 μM lactacystin in 10 mM Tris-HCl, 1 mM EDTA

(pH 8). The solution was then diluted tenfold with 20% aqueous acetonitrile containing 0.1% trifluoroacetic acid (TFA) and allowed to stand at room temperature for 5 min. Reverse-phase HPLC was then performed at room temperature using a Vydac C4 column (100 Å/4.6 x 150 mm) with 20-40% acetonitrile/0.1% TFA over ten minutes and then 40-55% acetonitrile/0.1% TFA over 30 min to elute the proteasome subunits (flow rate = 0.8 ml/min). Peaks were collected based on ultraviolet light absorbance at 210 and 280 nm, with the primary lactacystin-modified protein being the second to elute off the column and the secondary lactacystin-binding protein being the first. Protein from repeated injections was pooled, lyophilized and subjected to Edman degradation following tryptic digestion and reverse-phase HPLC separation of tryptic fragments. The putative lactacystin-modified residue was identified by adding subunit X/MB1 isolated from [^{3}H]lactacystin-treated proteasome to a sample that had been treated with unlabeled lactacystin, and then isolating and sequencing radioactive tryptic fragments. At one position, the phenylthiohydantoin-amino acid derivative was not identifiable, indicating possible modification of the residue.

Sequences from direct N-terminal sequencing and from tryptic fragments derived from the primary bovine lactacystin-binding protein aligned with sequences of human proteasome subunit X (predicted from the cDNA clone), and human LMP7-E2 (predicted from the exon 2-containing cDNA clone). Sequence 1 (from direct N-terminal sequencing) is also aligned with sequences of *Saccharomyces cerevisiae* Pre-2 (predicted from the genomic clone) and *Thermoplasma acidophilum* β-subunit (predicted from the cloned gene). The N-terminal heptapeptide corresponds to the tryptic fragment that appears to contain a lactacystin-modified N-terminal

threonine residue. The threonine at this position also corresponds to the putative N-termini of the mature, processed forms of all the homologs listed. Upper-case letters denote high confidence sequence, while lower case letters indicate lower confidence assignments.

Table 1

DIRECT N-TERMINAL SEQUENCES

1. Direct N-terminal sequence of primary bovine lactacystin-binding protein		TTTLAFKFRHggIIA		SEQ ID NO: 1
Human subunit X/MB1	5	TTTLAFKFRHGVIVA	19	SEQ ID NO: 2
Human LMP7-E2	74	TTTLAFKFQHGVIAA	88	SEQ ID NO: 3
S. cerevisiae Pre-2	76	TTTLAFRFQGGIIVA	90	SEQ ID NO: 4
T. acidopholum β-subunit	9	TTTVGITLKDAVIMA	23	SEQ ID NO: 5
2. Primary bovine lactacystin-binding protein		DAYSGGSVSLY		SEQ ID NO: 6
Human subunit X	171	DAYSGGAVNLYHVR	184	SEQ ID NO: 7
Human LMP7-E2	239	DSYSGGVVNMYHMK	252	SEQ ID NO: 8
3. Primary bovine lactacystin-binding protein		VIEINPYLLGTMAGGAADCSF		SEQ ID NO: 9
Human subunit X	38	VIEINPYLLGTLAGGAADCQFWER	61	SEQ ID NO: 10
Human LMP7-E2	106	VIEINPYLLGTMSGCAADCQYWER	129	SEQ ID NO: 11
4. Primary bovine lactacystin-binding protein		GYSYDLEVEEAYDLAR		SEQ ID NO: 12
Human subunit X	146	GYSDLEVEQAYDLAR	161	SEQ ID NO: 13
Human LMP7-E2	214	GYRPNLSPEEAYDLGR	229	SEQ ID NO: 14

Sequences of tryptic fragments derived from the secondary lactacystin-binding protein aligned with N-terminal fragment sequence of human erythrocyte proteasome α chain and of rat liver proteasome chain.

Table 2
TRYPTIC FRAGMENT SEQUENCES

Secondary bovine lactacystin-binding activity		
	TTIAGVVYK DGIVLGADTR	SEQ ID NO: 15
Human erythrocyte proteasome α chain		
	1 XXIAGVVYK DGIVLGADTR 19	SEQ ID NO: 16
Rat liver proteasome chain 1		
	1 TTIAGVVYK DGI 12	SEQ ID NO: 17

Sequence analysis of tryptic fragments derived from this protein showed it to be homologous to the proteasome α chain, a ~30 kDa protein identified in purified human erythrocyte proteasome and rat liver proteasome for which only an N-terminal fragment sequence exists. The second peak to elute contained only a ~24 kDa protein, the primary lactacystin-binding protein. Sequence from the N-terminus of the protein and from derived tryptic fragments showed high identity to the recently discovered 20S proteasome subunit X, also known as MB1, a homolog of the major histocompatibility complex (MHC)-encoded LMP7 proteasome subunit.

Example 5

Kinetics of inhibition of 20S proteasome peptidase activities

Experiments involving the proteasome were performed as follows: 20S proteasome (~5 ng/μl) in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA was incubated at 25°C in the presence of lactacystin or lactacystin analogs in DMSO or MeOH [not exceeding 5% (v/v)]. Aliquots for fluorescence assay were removed at various times following addition of compound and diluted in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA containing fluorogenic peptide substrates (100 μM final). Suc-LLVY-AMC, Cbz-GGR-βNA and Cbz-LLE-βA (AMC = 7-amido-4-methylcoumarin; βNA = β-naphthylamide) were used to assay for the chymotrypsin-like, trypsin-like and peptidylglutamyl-peptide hydrolyzing activities, respectively.

Biological activity of compound refers to the ability of the compound to induce neurite outgrowth in Neuro 2A neuroblastoma cells and to inhibit cell cycle progression in MG-63 osteosarcoma cells.

Table 3
Kinetics of Inhibition

		$\kappa_{assoc} = \kappa_{obs}/[I]$ $(s^{-1}M^{-1})$		
Compound (concentration)	Biological activity of compound	Chymotrypsin-like activity (Suc-LLVY-AMC)	Trypsin-like activity (Cbz-GGR-βNA)	Peptidylglutamyl-peptide hydrolyzing activity (Cbz-LLE-βNA)
Lactacystin (10 μM)	+	194±15	10.1±1.8	
Lactacystin (100 μM)	+			4.2±0.6
β-Lactone (1 μM)	+	3059±478		
β-Lactone (5 μM)	+		208±21	
β-Lactone (50 μM)	+			59±17
Dihydroxy acid (100 μM)	-	No inhibition	No inhibition	No inhibition
Lactacystin amide (12.5 μM)	+	306±99		
Phenylacetyl lactacystin (12.5 μM)	+	179±19		
6-Deoxylactacystin (12.5 μM)	-	No inhibition		
(6R,7S)Lactacystin (6-epi,7-epi) (12.5 μM)	-	No inhibition		
Des(hydroxyisobutyl)-lactacystin (12.5 μM)	-	No inhibition		

Example 6

Selectivity of protease inhibition

Experiments involving the other proteases were similar to Example 5, except that buffers used for incubation with lactacystin were as follows: α-Chymotrypsin: 10 mM Tris-HCL, pH 8, 1 mM EDTA (plus 100 μM Suc-LLVY-AMC for fluorescence assay); Trypsin: 10 mM

Tris-HCL, pH 8, 20 mM $CaCl_2$ (plus 100 μM Cbz-GGR-βNA for assay); Calpain I: 20 mM Tris-HCL, pH 8, 1 mM $CaCl_2$, 1 mM DTT (plus 100 μM Suc-LLVY-AMC for assay); Calpain II: 20 mM Tris-HCL, pH 8, 10 mM $CaCl_2$, 1 mM DTT (plus 100 μM Suc-LLVY-AMC for assay); Papain: 50 mM MES-NaOH, pH 6.4, 1 mM DTT (plus 100 μM Cbz-RR-AMC for assay); Cathepsin B: 100 mM KH_2PO_4, pH 5.5, 2 mM EDTA, 1 mM DTT (plus 100 μM Cbz-RR-AMC for assay). Fluorescent emission at 460 nm with excitation at 380 nm was measured for AMC-containing substrates, and emission at 410 nm with excitation at 335 nm was measured for βNA-containing substrates. The fluorescence assays were conducted at 25°C, each experiment was performed at least three times, and values represent mean ± standard deviation.

Table 4

Inhibition of Other Proteases

Protease tested	Effect of lactacystin (100 μM)
α-Chymotrypsin	No inhibition
Trypsin	No inhibition
Calpain I	No inhibition
Calpain II	No inhibition
Papain	No inhibition
Cathepsin B	No inhibition

The effects of lactacystin and the β-lactone on proteasame peptitase on activities using fluorogenic peptide substrates were assessed. All three peptidase activities were inhibited, irreversibly in the case of the trypsin-like and chymotrypsin-like activities and reversibly in the case of the PGPH activity. The

apparent second-order rate constant for association of each inhibitor with the enzyme, k_{assoc}, was determined for each of the activities (Table 1A). Lactacystin inhibits the chymotrypsin-like activity the fastest (k_{assoc} = 194 ± 15 M-1_s-1), the trypsin-like activity 20-fold more slowly, and the PGPH activity 50-fold more slowly. The fact that the inhibition kinetics are different for the three activities lends further support to the notion that the activities are due to separate active sites. The β-lactone displays the same rank order but inhibits each activity 15-20 times faster than does lactacystin itself, in accord with the greater expected chemical reactivity of the β-lactone. It is also possible that, upon initial binding to the protein target, the lactacystin thioester is cyclized in a rate-limiting step to the β-lactone, which serves as an activated intermediate for attack by the nucleophile.

The reversibility of the inhibitory effects was assessed by measuring residual peptidase activity after removal of excess inhibitor by extensive serial dilution/ultrafiltration. The trypsin-like and chymotrypsin-like activities were still completely inhibited in the lactacystin-treated samples following dilution/ultrafiltration, implying very low k_{off} of inhibitor from enzyme, whereas controls untreated with inhibitor maintained activity (data not shown). In the case of the PGPH activity, removal of the inhibitor was accompanied by a return of the catalytic activity; inhibition of the PGPH activity could be due to non-covalent association of lactacystin with the PGPH site or covalent association with turnover.

Previously, we had shown that the ability of analogs to cause neurite outgrowth in Neuro 2A cells was mirrored by their ability to inhibit cell-cycle progression in MG-63 osteosarcoma cells, and that

modifications to the γ-lactam part of the molecule impared both activities whereas modifications of the *N*-acetylcysteine moiety had little effect [Fenteany, 1994 3135]. We therefore tested the ability of the analogs to inhibit the 20S proteasome, but as our supplies of these materials were insufficient to examine all three activities, we focused on the chymotrypsin-like activity, which is inhibited most rapidly by lactacystin. The same trends found in the biological studies were apparent: the biologically active compounds inhibited the chymotryptsin-like activity about as well as lactacystin itself, and the biologically inactive compounds did not inhibit this activity as all (Table 3). Modifications of the γ-lactam core of lactacystin mitigate its effect, but modifications of the *N*-acetylcysteine moiety do not.

Example 7

Assay of the ability of lactacystin (β-lactone form) can block TNF-α dependent degradation of IκB-α *in vivo.*

Hela cells were plated onto 6-well plates in DME plus 10% fetal calf serum (3 mls/well). Cells were then pretreated with 0.125% DMSO 40 μM G132 (carbobenzoxyl-leucinyl-leucinyl-leucinal-H)(40 μM, lanes 103), or 5 μM β-lactone for one hour, followed by treatment with 1,000 U/ml TNF-α or phosphate buffered saline (PBS). Cells were harvested after 0 min, 20 min, or 40 min of further incubation at 37°C. Cells were then lysed in buffer containing NP-40 and protease inhibitors, and the proteins separated on a 10% SDS-polyacrylamide gel. The proteins were then transferred to nitrocellulose and probed with antibodies against the C-terminal 20 amino acids of human IκB-α.

Previous reports have shown that the inducible degradation of IκB is preceded by phosphorylation of the protein (Beg *et al.*, 1993, *Mol. Cell. Biol.* 13:3301;

Traenckner *et al.*, 1994, EMBO J. 13:5433; Miyamoto *et al.*, 1994, PNAS 91:12740; Lin *et al.*, 1995, PNAS 92:552; DiDonato *et al.*, 1995, Mol. Cell. Biol. 15:1302; and Alkalay *et al.*, 1995, Mol. Cell. Biol. 15:1294). The phosphorylated IκB is then preferentially degraded, apparently by the proteasome (Palombella *et al.*, 1994, Cell 78:773). Phosphorylation is evidenced by a slightly heavier shift in the electrophoretic mobility of IκB. IκB from cells induced with TNF show the requisite pattern of phosphorylation and degradation over time. Cells treated with PBS only show no change in the level or modification of IκB. As was reported previously, (Palombella et al, 1994), the peptide aldehyde proteasome inhibitor MG132 blocks the degradation of IκB and stabilizes the phosphorylated form. β-lactone also blocks degradation and stabilizes the phosphorylated form of IκB. These results indicate that lactacystin does not effect the TNF dependent signalling pathway that leads to the phosphorylation of IκB, but does block the degradation of the protein. Lactacystin inhibits the degradation of IκB after TNF induction of Hela cells, most likely by specifically blocking the action of the proteasome.

Example 8

<u>Assay of the effect of lactacystin (β-lactone form) on the proteasome dependent p105 to p50 processing *in vivo*.</u>

COS cells were plated onto 100 mm dishes, then, with DEAE-Destran, either mock transfected, or transfected with 3 μg of pcDNA plasmid containing the human p105 cDNA. Forty-eight hrs after transfection, cells were pretreated for 1 hour with 0.5% DMSO, 50 μM calpain inhibitor II, 50 μM MG132, or 10 μM β-lactone. Cells were then pulse labelled with 250 uCi/plate of ^{35}S-

methionine/cysteine for 15 minutes, and either harvested immediately or followed by a 2 hour chase with excess unlabelled methionine and cysteine. Cells were then lysed with SDS/Tris, and proteins were immunoprecipitated with anti-p50 antibodies (recognize the *N*-terminal half of the p105 protein). These proteins were then separated on a 10% SDS-polyacrylamide gel, which was then fixed, dried, and exposed to autoradiographic film.

An analysis of the proteins isolated immediately after the pulse-labelling period reveals significant amounts of labelled p105 protein and very little p50. After the 2 hour chase period, the levels of p105 were reduced, and a new band that corresponds to p50 protein is apparent, as was expected (Fan and Maniatis, 1991, *Nature* 354:395; Palombella *et al.*, 1994, *Cell* 78:773). Pretreatment of cells with calpain inhibitor II has no effect on the processing of p105 to p50 (lane 4). However, treatment of cells with the peptide aldehyde proteasome inhibitor MG132 completely blocks the appearance of p50 (lane 5), as has been reported previously (Palombella *et al.*, 1994). Low levels of β-lactone (10 μM) have only a slight effect on the level of p50, but higher concentrations (50 μM) result in the complete inhibition of the processing of p105 to p50. Lactacystin efficiently blocks the proteasome dependent procession of p105 to p50 *in vivo*.

Example 9

Neurite Outgrowth Assay

Compounds are dissolved in the minimal amount of methanol (MeOH) or dimethyl sulfoxide (DMSO) required for solubilization. No more than 0.1% solvent is present in any assay. When necessary, solutions are evaporated to dryness and resuspended in cell culture medium to their final concentrations before use.

Neuro 2A, IMR-32, PC12, and MG-63 cells are obtained from the American Type Culture Collection. Neuro 2A and IMR-32 cells are cultured in Eagle's minimal essential medium (MEM) containing 10% (vol/vol) fetal bovine serum (FBS). PC12 cells are grown in RPMI 1640 medium containing 10% (vol/vol) horse serum and 5% FBS, and MG-63 cells are cultured in RPMI 1640 containing 10% FBS.

Neuro 2A cells are plated at a density of 1 x 10^4 cells per 1 ml per well in 12-well polystyrene dishes (22-mm-diameter flat-bottom wells) and are grown for 24 h in MEM with 10% FBS prior to any treatment. In the relevant experiments, nocodazole, cytochalasin B, or cycloheximide is added 3 h before addition of lactacystin. In the serum deprivation experiments, cells are switched to serum-free MEM 24 h after plating and, when relevant, incubated another 24 h before addition of lactacystin and subsequently maintained in serum-free conditions.

Example 10

<u>Cell Cycle Analysis</u>

MG-63 cells are plated at 7.5 x 10^4 cells per 3 ml per 25-cm^2 flask and grown for 24 h in RPMI containing 10% FBS. These subconfluent MG-63 cultures are synchronized in G_0/G_1 by changing the medium to RPMI containing 0.2% FBS and incubating for 64 h. This is followed by stimulation with 2 ml of RPMI containing 10% FBS and addition of compounds. Neuro 2A cells are grown to ~2 x 10^7 in 175-cm^2 flasks in MEM with 10% FBS. Mitotic cells are harvested by shaking for 5 min at 100 rpm on a rotary shaker. The detached cells are replated at 1.5 x 10^5 cells per 2 ml per 25-cm^2 flask and incubated for 30 min to allow for reattachment prior to addition of lactacystin. Cells are harvested for cell cycle analysis

21 h after stimulation in the case of the MG-63 cells and 20 h after replating in the case of the Neuro 2A cells and then were processed for flow cytometry. DNA histograms are obtained using a Becton Dickinson FACScan flow cytometer.

Example 11

Purification of 20S Proteasome and Proteasome Activator PA28

20S proteasome was purified by a modification of procedures by Hough et al. [Hough, R., et al., (1987) *J. Biol. Chem.*, 262, 8303-8313] and Ganoth et al. [Ganoth, D., et al., (1988) *J. Biol. Chem.*, 263, 12412-12419. PA28 Activator was purified by a modification of procedures of Chu-Ping et al. [Chu-Ping, M., et al., (1992) *J. Biol. Chem.*, 267, 10515-10523]. Rabbit reticulocytes were obtained from Green Hectares (Oregon, WI). The reticulocytes were washed three times by suspending them in ice cold PBS and centrifuging at 2000 xg for 15 min. After the final wash, the cells were lysed in 1.5 volumes of cold distilled water containing 1 mM DTT. The mixture was then passed through a Glas-CO Bio-Nebulizer at 250 psi to ensure complete cell lysis. The lysate was then centrifuged at 100,000 xg for one hour to remove cell debris. The supernatant (extract) was brought to 20 mM HEPES pH 7.6, 1 mM DTT (Buffer A), filtered through a 0.2 μm filter, and applied to a DE52 anion exchange column equilibrated in Buffer A. The column was washed with 5 volumes of Buffer A and absorbed proteins were eluted with 2.5 volumes of Buffer A containing 500 mM NaCl. The eluate (Fraction II) was brought to 38% saturation with ammonium sulfate and centrifuged at 10,000 xg for 20 minutes. The supernatant was brought to 85% saturation with ammonium sulfate and centrifuged 10,000 xg for 20 minutes. The resulting

pellets were resuspended in a minimal volume of Buffer A and dialyzed vs. 4L of Buffer A overnight. The dialysate (Fraction IIB), which contains both 20S proteasome and PA28 activator, was applied to a Mono Q anion exchange column. Elution was performed by a 40 column volume gradient from 0-500 mM NaCl.

20S Proteasome

Column fractions were assayed for SDS-stimulated Suc-Leu-Leu-Val-Tyr-AMC hydrolyzing activity. The active fractions were pooled, diluted to 50 mM [NaCl], and applied to a 1 mL Heparin Sephrose Hi-Trap column. Elution was performed with a 20 column volume gradient from 50 mM-500 mM NaCl. Active fractions were pooled and concentrated using a 30,000 MWCO Centricon centrifugal concentrator. The concentrate was applied to a Superose 6 size exclusion chromatography column and eluted with Buffer A containing 100 mM NaCl. The active fractions that were judged to be pure by SDS-PAGE analysis were pooled and stored at -80° C for subsequent use.

PA28

Column fractions (vide supra) were assayed for their ability to stimulate the Suc-Leu-Leu-Val-Tyr-AMC hydrolysing activity of exogenous 20S proteasome. The active fractions were pooled and diluted to 10 mM NaCl. This material was then further purified and concentrated on a 1 ml Resource Q anion exchange column with a 40 column volume gradient from 10-500 mM NaCl. Active fractions were pooled and concentrated using a 10,000 MWCO Centricon centrifugal concentrator. The concentrate was applied to a Superdex 200 size exclusion chromatography column and eluted with Buffer A containing 100 mM NaCl. The active fractions that were judged to be pure by SDS-PAGE analysis were pooled and stored at -80° C for subsequent use.

Example 12

Lactacystin Inactivation of Proteasome Activity

2 ml of assay buffer (20 mM HEPES, 0.5 mM EDTA, pH 8.0) and Suc-Leu-Leu-Val-Tyr-AMC in DMSO were added to a 3 ml fluorescent cuvette and the cuvette was placed in the jacketed cell holder of a Hitachi F-2000 fluorescence spectrophotometer. The temperature was maintained at 37°C by a circulating water bath. 0.34 μg of proteasome and 3.5 μg of PA28 were added and the reaction progress was monitored by the increase in fluorescence at 440 nm (λex = 380 nm) that accompanies production of free AMC. The progress curves exhibited a lag-phase lasting 1-2 minutes resulting from the slow formation of the 20S-PA28 complex. After reaching a steady-state of substrate hydrolysis, lactacystin was added to a final concentration of 1 μM and the reaction was monitored for 1 hr. The fluorescence (F) versus time (t) data were collected on a microcomputer using LAB CALC (Galactic) software. k_{inact} values were estimated by a nonlinear least-squares fit of the data to the first-order equation:

$$F = A(1-e^{-kt}) + C$$

where $C = F_{t=0}$ and $A = F_{t=\infty} - F_{t=0}$. Owing to the complex kinetics of lactacystin hydrolysis and its mechanism of inactivation of the proteasome the kinetics of proteasome inactivation are also complex and this fact is reflected in the curve fitting. There was a small systematic deviation of the measured progress curves from the "best-fit" curves generated by fitting the data to the equation above. Estimates of k_{inact} were obtained using this simple kinetic model.

Example 13

Inhibition of Protein Degradation in C2C12 Cells

C2C12 cells (a mouse myoblast line) were labeled for 48 hrs with ^{35}S-methionine. The cells were then washed and preincubated for 2 hrs in the same media supplemented with 2 mM unlabeled methionine. The media was removed and replaced with a fresh aliquot of the preincubation media containing 50% serum, and a concentration of the compound to be tested. The media was then removed and made up to 10% TCA and centrifuged. The TCA soluble radioactivity was counted. Inhibition of proteolysis was calculated as the percent decrease in TCA soluble radioactivity. From this data, an IC_{50} for each compound was calculated. The compounds can be qualitatively grouped into ++, +, and 0 categories, wherein wherein ++ indicates better activity than +, and 0 indicates little or no activity, due to insolubility, instability, or other reasons. For example, in the lactacystin group compounds I-1, I-2, and I-3 were in the ++ category; compound I-4 was in the + category. Turning to the P series of lactone-like compounds, P-1 through P-4 were in the ++ category; compounds P-5 and P-6 were in the + category; and compound P-7 was in the 0 category.

Example 14

Synthesis of Compound I-1

(3R,4S,5R,1'S)-4-Hydroxy-5(1'-hydroxy-2'-methylpropyl)-3-methylpyrrolidin-2-one-5-carboxylic acid (10.0 mg, 43.2 mmol; made by the route described by Corey, E. J.; Reichard, G. A., *J. Am. Chem. Soc.* **1992**, *114*, 10677) was dissolved in 0.4 mL anhydrous dichloromethane and then treated, in order, with tri-ethylamine (18.0 mL, 0.13 mmol, 3.0 equiv.), 13.0 mg bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (51.1 mmol, 1.2 equiv.) and benzyl

mercaptan (8.0 mL, 68.1 mmol, 1.58 equiv.). The solution was stirred for 5 hours at room temperature and then diluted with dichloromethane and washed with water. The aqueous layer was extracted with ethyl acetate and the organic layers were combined and dried over magnesium sulfate. Filtration followed by removal of the solvent afforded a crude solid which was triturated with 19:1 hexane/ethyl acetate and collected by filtration. The white solid obtained was then dissolved in a minimum amount of acetonitrile and the clear solution was diluted with water then frozen and lyophilized overnight. This afforded 8.0 mg of the desired thioester I-1 (55%) as a fluffly white solid. ^1H NMR (DMSO-d_6, 300 MHz) d 8.34 (s, 1H, NH), 7.19-7.28 (m, 5H), 5.40 (d, 1H, ^3J= 6.4 Hz, OH), 5.11 (d, 1H, ^3J = 7.4 Hz, OH), 4.39 (t, ^3J = 6.4 Hz), 4.04 (s, 2H), 3.76 (t, 1H, ^3J = 7.4 Hz), 2.62 (m, 1H), 1.55 (m, 1H), 0.90 (d, 3H, ^3J = 7.5 Hz), 0.85 (d, 3H, ^3J = 6.6 Hz), 0.66 (d, 3H, ^3J = 6.8 Hz).

Example 15

Synthesis of Compound I-2

To a solution of glutathione (360 mg, 1.17 mmol) in 11 mL of water was added 1.35 mL of 1.004 N sodium hydroxide in water. This solution was then added to a freshly prepared solution of *clasto*-lactacystin-b-lactone (49.0 mg, 0.23 mmol; made by the route described by Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677) in 10 mL of acetonitrile. After stirring the solution at room temperature for 4 hours, the pH was adjusted to about pH = 4 by the slow addition of 1 N aqueous hydrochloric acid solution. The acetonitrile was removed *in vacuo* and the aqueous phase was frozen and lyophilized. The solid obtained was redissolved in water, filtered and lyophilized again. The white solid obtained was purified by reverse phase HPLC [Waters Prep Delta-Pak® 19 x 300 mm C18 column

eluting with 98% 0.05% trifluoroacetic acid/H_2O and 2% 0.05% trifluoroacetic acid/methanol to 80% 0.05% trifluoroacetic acid/H_2O and 20% 0.05% trifluoroacetic acid/methanol in 65 min at 5 mL/min]. After lyophilizing the pure fractions, glutathione thioester adduct **I-2** (48 mg, 40%) was obtained as a fluffly white solid. 1H NMR (DMSO-d6, 300 MHz) d 7.73-8.26 (m, 4H), 5.28 (bs, 1H, OH), 5.09 (bs, 1H, OH), 4.44 (m, 1H), 4.37 (bd, 1H, $3J$ = 5.9 Hz), 3.74 (m, 2H), 3.23 (dd, 1H, J = 13.3, 4.6 Hz), 2.95 (dd, 1H, J = 13.3, 8.8 Hz), 2.62 (m, 1H), 2.29-2.32 (m, 2H), 1.98-2.08 (m, 2H), 1.51-1.63 (m, 1H), 0.92 (d, 3H, ^3J = 7.5 Hz), 0.86 (d, 3H, $3J$ = 6.6 Hz), 0.71 (d, 3H, ^3J = 6.7 Hz).

Example 16

Synthesis of Compound I-4

(3R,4S,5R,1'S)-4-Hydroxy-5(1'-hydroxy-2'-methylpropyl)-3-methylpyrrolidin-2-one-5-carboxylic acid (20.0 mg, 86.5 mmol; made by the route described by Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677) was dissolved in 1.0 mL acetonitrile and then treated, at 0°C, with 1,8-diazabicyclo[5.4.0]undec-7-ene (14.0 mL, 93.6 mmol, 1.08 equiv.) followed by benzyl bromide (12.0 mL, 101 mmol, 1.17 equiv.). The mixture was stirred at room temperature overnight and was then partitioned between ethyl acetate and water. The aqueous layer was extracted three times with ethyl acetate and the organic layers were then combined and dried over magnesium sulfate. Filtration followed by removal of the solvent afforded a crude solid which was triturated with hexane and collected by filtration. The white solid obtained was then dissolved in a minimum amount of acetonitrile and the clear solution was diluted with water then frozen and lyophilized overnight. This afforded 6.7 mg of the desired ester **I-4** (24%) as a fluffly white solid. 1H NMR (DMSO-d_6, 300 MHz) d 8.02 (s, 1H, NH), 7.31-7.44 (m, 5H), 5.45 (d, 1H, $3J$= 6.4 Hz,

OH), 5.11 (d, 1H, ^2J = 12.5 Hz), 5.03 (d, 1H, ^3J = 7.2 Hz, OH), 5.00 (d, 1H, ^2J = 12.5 Hz), 4.31 (t, ^3J = 6.4 Hz), 3.76 (t, 1H, ^3J = 7.2 Hz), 2.68 (m, 1H), 1.52 (m, 1H), 0.89 (d, 3H, ^3J = 7.4 Hz), 0.85 (d, 3H, ^3J = 6.6 Hz), 0.67 (d, 3H, ^3J = 6.7 Hz).

Example 17

Synthesis of Compound P-2

(3S,7aR)-3-Phenyl-5,6,7,7a-tetrahydro-*1H*-pyrrolo[1,2-c]oxazol-5-one was prepared in three steps from D-Pyroglutamic acid by a known procedure (Thottathil, J. K. et al. *J. Org. Chem.* **1986**, *51*, 3140) and was converted to (3S,7aR)-3-Phenyl-*1H*-pyrrolo[1,2-c]oxazol-5-one in two steps (selenylation and oxidative elimination) by the method reported by Hamada et al. (*J. Am. Chem. Soc.* **1989**, *111*, 1524). (3S,7aR)-3-Phenyl-*1H*-pyrrolo[1,2-c]oxazol-5-one was then converted to (3S,7S,7aS,1'S)-7a-(1'-acetoxy-2'-methylpropyl)-7-hydroxy-3-phenyl-5,6,7,7a-tetrahydro-*1H*-pyrrolo[1,2-c]oxazol-5-one in 6 steps by analogy to the sequence reported by Uno *et al.* (*J. Am. Chem. Soc.* **1994**, *116*, 2139). (3S,7S,7aS,1'S)-7a-(1'-acetoxy-2'-methylpropyl)-7-hydroxy-3-phenyl-5,6,7,7a-tetrahydro-*1H*-pyrrolo[1,2-c]oxazol-5-one (70 mg, 0.21 mmol) was dissolved in 1.0 mL pyridine and treated at room temperature with 32.0 mL acetic anhydride (0.34 mmol, 1.6 equiv.). The mixture was stirred at room temperature overnight and the solvent was removed *in vacuo* affording 77 mg of desired (3S,7S,7aS,1'S)-7a-(1'-acetoxy-2'-methylpropyl)-7-acetoxy-3-phenyl-5,6,7,7a-tetrahydro-*1H*-pyrrolo[1,2-c]oxazol-5-one (97%), a yellow oil which was used as such in the following step.

The crude (3S,7S,7aS,1'S)-7a-(1'-acetoxy-2'-methylpropyl)-7-acetoxy-3-phenyl-5,6,7,7a-tetrahydro-*1H*-pyrrolo[1,2-c]oxazol-5-one (75 mg, 0.20 mmol) was dissolved in 5 mL methanol and placed under 1 atmosphere of hydrogen in

the presence of 50 mg 20% palladium hydroxide on carbon. When a TLC analysis (1:1 hexane/ethyl acetate) showed the reaction to be complete, the mixture was filtered and concentrated *in vacuo* affording 55 mg (97%) of the desired primary alcohol which without any purification was converted to (4S,5R,1'S)-4-Hydroxy-5-(1'-hydroxy-2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic by a two step sequence (Jones' oxidation, saponification) analogous to the sequence reported by Uno *et al.* (*J. Am. Chem. Soc.* **1994**, *116*, 2139).

(4S,5R,1'S)-4-Hydroxy-5-(1'-hydroxy-2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid (7.0 mg, 32 mmol) was dissolved in 2.0 mL 1:1 acetonitrile/tetrahydrofuran and then treated, at room temperature, with 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium tetrafluoroborate (11.0 mg, 34 mmol, 1.1 equiv.) followed by triethylamine (0.36 mmol, 11 equiv.). The mixture was stirred for 30 min to room temperature and concentrated *in vacuo*. Flash chromatography (230-400 mesh SiO, elution with ethyl acetate) finally afforded 3 mg of the pure lactone **P-2** (47%) obtained as white solid. H NMR (DMSO-d6, 300 MHz) d 8.95 (bs, 1H, NH), 5.64 (d, 1H, *3J*= 6.7 Hz, OH), 5.26 (d, 1H, *3J* = 6.0 Hz, OH), 3.78 (dd, 1H, *3J*= 6.7, 3.5 Hz), 2.81 (dd, 1H, *2J*= 19.2 Hz, *3J*= 6.0 Hz), 2.60 (d, 1H, *2J*= 19.2 Hz), 1.74 (m, 1H), 0.90 (d, 3H, *3J* = 6.9 Hz), 0.68 (d, 3H, ^3J = 6.8 Hz).

Example 18

Synthesis of Compound P-3

Clasto-Lactacystin-β-lactone (5.0 mg, 23.4 mmol; made by the route described by Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677) was dissolved in 1.5 mL pyridine and treated, at room temperature with acetic anhydride (40 mL, 0.42 mmol, 18 equiv.). After stirring the solution at room temperature overnight, the pyridine was removed *in vacuo* with the aid of 2 mL of toluene affording a light brown solid. The solid was washed with three portions of hexane and then filtered giving 5.5 mg of the pure acetate **P-3** (93%) obtained as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz) d 9.11 (bs, 1H, NH), 5.33 (d, 1H, ^3J= 6.1 Hz), 5.21 (d, 1H, ^3J = 5.0 Hz), 2.84 (m, 1H), 2.06 (s, 3H), 1.90 (m, 1H), 1.08 (d, 3H, ^3J = 7.5 Hz), 0.87 (d, 3H, ^3J = 6.9 Hz), 0.78 (d, 3H, ^3J = 6.8 Hz).

Example 19

Synthesis of Compound P-4

(3S,7aR)-3-Phenyl-5,6,7,7a-tetrahydro-*1H*-pyrrolo[1,2-c]oxazol-5-one was prepared in three steps from D-Pyroglutamic acid by a know procedure (Thottathil, J. K. et al. *J. Org. Chem.* **1986**, *51*, 3140) and was converted to (3S,7aR)-3-Phenyl-*1H*-pyrrolo[1,2-c]oxazol-5-one in two steps (selenylation, and oxidative elimination) by the method reported by Hamada et al. (*J. Am. Chem. Soc.* **1989**, *111*, 1524). (3S,7aR)-3-Phenyl-*1H*-pyrrolo[1,2-c]oxazol-5-one was then converted to (3S,6R,7S,7aS,1'S)-7a-(1'-acetoxy-2'-methylpropyl)-6-hydroxy-7-hydroxy-3-phenyl-5,6,7,7a-tetrahydro-*1H*-pyrrolo[1,2-c]oxazol-5-one in 4 steps by analogy to the sequence reported by Uno *et al.* (*J. Am. Chem. Soc.* **1994**, *116*, 2139).

(3S,6R,7S,7aS,1'S)-7a-(1'-acetoxy-2'-methylpropyl)-6-hydroxy-7-hydroxy-3-phenyl-5,6,7,7a-tetrahydro-*1H*-pyrrolo[1,2-c]oxazol-5-one was then converted in 4 steps

(acetylation, hydrogenolysis, oxidation and saponification) to(3R,4S,5R,1' S)-4-Hydroxy-5-(1' -hydroxy-2' -methylpropyl)-3-hydroxypyrrolidin-2-one-5-carboxylic acid in the same manner described for making P-2.

(3R,4S,5R,1' S)-4-Hydroxy-5-(1' -hydroxy-2' -methyl-propyl)-3-hydroxypyrrolidin-2-one-5-carboxylic acid (20 mg, 86 mmol) was converted to the corresponding β-lactone by the method described for making P-2. This afforded 3 mg of the pure lactone **P-4** (16%) obtained as white solid. H NMR (DMSO-d_6, 300 MHz) d 8.96 (s, 1H, NH), 6.08 (bs, 1H, OH), 5.63 (d, 1H, ^3J= 6.6 Hz, OH), 5.12 (d, 1H, ^3J = 5.7 Hz), 4.31 (d, 1H, ^3J = 5.7 Hz), 3.72 (bt, 1H), 1.70 (m, 1H), 0.88 (d, 3H, ^3J = 6.9 Hz), 0.72 (d, 3H, ^3J = 6.8 Hz).

Example 20

Synthesis of Compound P-6

Clasto-Lactacystin-β-lactone (11.8 mg, 55.3 mmol; made by the route described by Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677) was dissolved in 0.4 mL freshly distilled pyridine and treated, at room temperature with methanesulfonyl chloride (6.0 mL, 77.5 mmol, 1.4 equiv.). The solution was stirred for 2 hours at room temperature, treated with more methanesulfonyl chloride (10.0 mL, 129 mmol, 2.3 equiv.) and stirred at room temperature overnight. The dark solution was then concentrated *in vacuo* with 3 x 2 mL of toluene affording a yellow solid. Flash chromatography (230-400 mesh SiO_2, elution with 1:1 hexane/ethyl acetate) finally afforded 12.6 mg of the pure mesylate **P-6** (78%) obtained as fluffy white solid. ^{1}H NMR ($CDCl_3$, 300 MHz) d 6.28 (bs, 1H, NH), 5.27 (d, 1H, ^3J= 6.1 Hz), 5.06 (d, 1H, ^3J = 4.0 Hz), 3.11 (s, 3H), 2.80-2.89 (m, 1H), 2.24-2.34 (m, 1H), 1.33 (d, 3H, ^3J = 7.5 Hz), 1.14 (d, 3H, ^3J = 7.0 Hz), 1.05 (d, 3H, ^3J = 6.8 Hz).

OTHER EMBODIMENTS

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: President and Fellows of Harvard College

(ii) TITLE OF INVENTION: LACTACYSTIN ANALOGS

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US96/
(B) FILING DATE: 12-APR-1996
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/421,583
(B) FILING DATE: 12-APR-1995

(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: 00246/197W01

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(B) TELEFAX: 617/542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Thr Thr Thr Leu Ala Phe Lys Phe Arg His Gly Gly Ile Ile Ala
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Thr Thr Thr Leu Ala Phe Lys Phe Arg His Gly Val Ile Val Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Thr Thr Thr Leu Ala Phe Lys Phe Gln His Gly Val Ile Ala Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Thr Thr Thr Leu Ala Phe Arg Phe Gln Gly Gly Ile Ile Val Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Thr Thr Thr Val Gly Ile Thr Leu Lys Asp Ala Val Ile Met Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Ala Tyr Ser Gly Gly Ser Val Ser Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Ala Tyr Ser Gly Gly Ala Val Asn Leu Tyr His Val Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asp Ser Tyr Ser Gly Gly Val Val Asn Met Tyr His Met Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Val Ile Glu Ile Asn Pro Tyr Leu Leu Gly Thr Met Ala Gly Gly Ala
1               5                   10                  15

Ala Asp Cys Ser Phe
            20
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Val Ile Glu Ile Asn Pro Tyr Leu Leu Gly Thr Leu Ala Gly Gly Ala
1               5                   10                  15

Ala Asp Cys Gln Phe Trp Glu Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Val Ile Glu Ile Asn Pro Pro Tyr Leu Leu Gly Thr Met Ser Gly Cys
1               5                   10                  15

Ala Ala Asp Cys Gln Tyr Trp Glu Arg
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gly Tyr Ser Tyr Asp Leu Glu Val Glu Glu Ala Tyr Asp Leu Ala Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gly Tyr Ser Asp Leu Glu Val Glu Gln Ala Tyr Asp Leu Ala Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Tyr Arg Pro Asn Leu Ser Pro Glu Glu Ala Tyr Asp Leu Gly Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Thr Thr Ile Ala Gly Val Val Tyr Lys Asp Gly Ile Val Leu Gly Ala
1               5                   10                  15

Asp Thr Arg
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Xaa Xaa Ile Ala Gly Val Val Tyr Lys Asp Gly Ile Val Leu Gly Ala
1               5                   10                  15

Asp Thr Arg
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

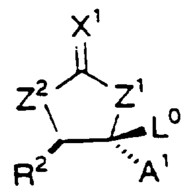
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Thr Thr Ile Ala Gly Val Val Tyr Lys
1               5
```

What is claimed is:

CLAIMS

1. A compound having the formula



wherein Z^1 is O, S, SO_2, NH, or NR_a, R_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} h eterocyclic radical, or halogen, X^1 being two singly bonded H when Z^1 is SO_2;

Z^2 is O, S, NH, NR_d, CHR^1, or $CHOR^1$ in the *(R)* or *(S)* configuration, wherein R_d is C_{1-6} alkyl and R^1 is H, halogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, NR_dR_e (except where Z^2 is $CHOR^1$), or the side chain of any naturally occurring α-amino acid, or R^1 and R^2 taken together are a bivalent moiety, provided that when R^1 and R^2 are taken together, Z^1 is NH or NR_a and Z^2 is CHR^1; R_e being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, and the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl, where the H in CHR^1 is deleted when R_1 and R_2 taken together form a C_{3-8} heteroaryl or C_{6-12} aryl;

R^2 is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, azido, C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H, C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1

and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl, where the H in CHR^1 is deleted when R_1 and R_2 taken together form a C_{3-8} heteroaryl or C_{6-12} aryl; and when R^1 and R^2 are taken together, Z^2 is CR^1, and one of A^1 and L^O is (C_{1-4} alkyl)oxycarbonyl or carboxyl, the other of A^1 and L^O has a fragment formula weight of at least 20;

A^1 is the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

L^O is $-(C=X^2)-L^1$, $-(C=X^2)-L^2$, or $-C(L^2)-CH_2-O$, wherein X^2 is O or S; L^2 is H, C_{1-6} haloalkyl, C_{1-6} alkyl, or C_{6-14} aryl, and L^1 is a moiety having 0 to 25 carbon atoms, 0 to 10 heteroatoms, and 0 to 6 halogen atoms, L^1 being linked by an oxygen or sulfur atom to the carbon atom bonded to X^2, where A^1 is C_{5-20} alkyl when L^O is carboxyl or (C_{1-4} alkyl)-oxy-carbonyl and A^1 is alkyl; where only one of A^1 and L^O is

selected from carboxyl and (C_{1-4} alkyl)oxycarbonyl; where L^1 has at least 3 carbon atoms when Z^1 and Z^2 are both NH, and A^1 is methoxy; and when Z^1 is NH and Z^2 is (*R*) CHR^1, L^1 has between 0 and 3 nonaromatic acyclic carbon atoms when there are 5 heteroatoms, L^1 has between 6 and 15 nonaromatic acyclic carbon atoms when there is only one oxygen atom, L^1 has 0, 1, 3, or 5 nonaromatic carbon atoms when there are three halogen atoms.

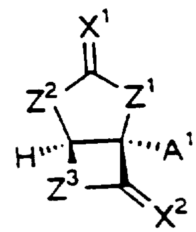
2. A compound of claim 1, wherein Z^2 is CHR^1 and Z^1 is NH or NR_a.

3. A compound of claim 1, wherein L^0 is C_{2-16} oxiranyl; L^1 is selected from hydroxy, C_{1-12} alkoxy, C_{1-12} alkylsulfonyloxy, C_{6-20} arylsulfonyloxy, C_{7-20} arylalkyl, C_{6-20} aryloxy, C_{6-20} arylalkylcarbonyloxy, C_{2-8} alkylcarbonyloxy, C_{6-20} arylalkylcarbonylthio, C_{6-20} arylthio, C_{2-8} alkylcarbonylthio, and C_{1-12} alkylthio; or L^2 is C_{1-2} haloalkyl, C_{1-6} alkyl, or H.

4. A compound of claim 1, wherein Z^2 is O, S, NH, or NR_d.

5. A compound of claim 1, wherein Z^2 is O, S, NH, NR_d, or CHR^1; and R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl.

6. A compound having the following formula



wherein Z^1 is O, S, SO_2, NH, or NR_a, R_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen, provided that when Z^1 is SO_2, X^1 is two singly bonded H;

Z^2 is CHR^1 in the *(R)* or *(S)* configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl,C_{2-6} alkenyl, or C_{2-5} alkynyl);

Z^3 is O, S, NH, or NR_j, R_j being C_{1-6} alkyl;

X^2 is O or S; and

A^1 is the side chain of any naturally occurring α-amino acid, or is of the following formula,

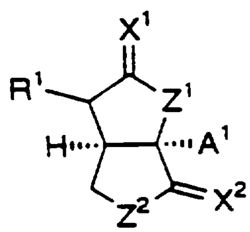
$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i-(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; each of R_i and $R_{i'}$, independently,

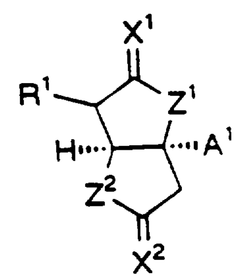
is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; provided that R^1 is H, OH or OR_d when X^4 is hydroxyl in the (S) configuration and $-(CH_2)_n-R^3X^3$ is isopropyl; and provided that the moiety $-(CH_2)_n-R^3X^3$ has between 5 and 20 carbon atoms when X^4 is hydroxyl in the (R) configuration, m is 0, and R^1 is C_{1-6} alkyl.

7. A compound of claim 6, wherein Z^1 is NH or NR_a.

8. A compound having one of the following formulae



or



wherein Z^1 is NH or NR_a, R_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen;

Z^2 is O, S, NH, or NR_j, R_j being C_{1-6} alkyl;

R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen);

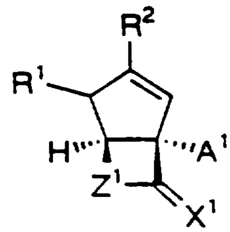
X^2 is O or S; and

A^1 is the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; when X^1 is 2 singly bonded H and R^1 has only one oxygen, then the fragment formula weight of R^1 is at least 30 atomic mass units; when X^1 is 2 singly bonded H and R^1 is 2 H, and A^1 is alkyl, then A^1 has at least 3 carbon atoms; A^1, R^1 and X^1 taken together have at least one carbon atom, halogen, or heteroatom; when X^1 is two singly bonded H, and R^1 is H, X^2 is O, and R_h is alkyl, then R_h is C_{4-6} alkyl; and when n is 2, R_h is C_{1-6} haloalkyl.

9. A compound having the following formula



wherein Z^2 is O, S, NH or NR_j, R_j being C_{1-6} alkyl;

X^1 is O or S;

R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl);

R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-12} aryl, C_{3-8} heteroaryl, or halogen;

X^2 is O or S; and

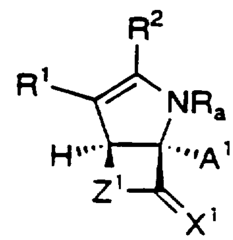
A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H,

hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

10. A compound having the following formula



wherein Z^1 is O, S, NH or NR_j, R_j being C_{1-6} alkyl;

X^1 is O or S;

R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen);

R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-12} aryl, C_{3-8} heteroaryl, or halogen;

R_a is C_{1-6} alkyl; and

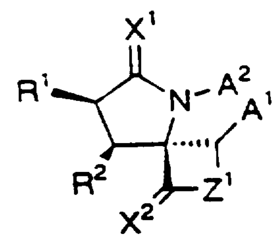
A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6}

alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

11. A compound having the formula



wherein X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen;

Z^1 is O, S, NH, or NR_a, R_a being C_{1-6} alkyl;

R^1 H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of any naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen); or R^1 and R^2 taken together are a bivalent moiety which forms a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl;

R^2 is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, azido,

C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H, C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1 and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl;

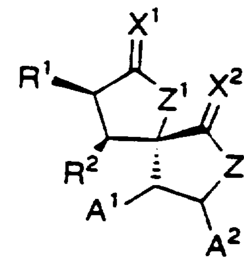
X^2 is O or S; and

A^1 is in the *(R)* or *(S)* configuration, and each of A^1 and A^2 is independently H, the side chain of any naturally occurring amino α-acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

12. A compound having the following formula



wherein Z^1 is NH or NR_a, NR_a being C_{1-6} alkyl; X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen;

Z^2 is O, S, NH, or NR_j, R_j being C_{1-6} alkyl;

R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of any naturally occurring amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen); or R^1 and R^2 taken together are a bivalent moiety which forms a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl;

R^2 is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, azido, C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H, C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1 and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl;

X^2 is O or S; and

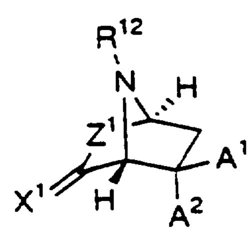
each of A^1 and A^2 is independently in the *(R)* or *(S)* configuration, and is independently H, the side chain of

any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

13. A compound of the formula



wherein Z^1 is O, NH, or NR_a, NR_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, or two singly bonded H;

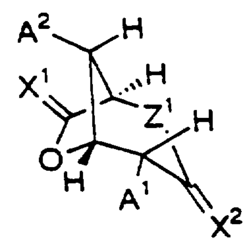
each of A^1 and A^2 is independently H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

R^{12} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl; where A^1, A^2, R^{12}, and X^1 taken together have at least one carbon atom and one heteroatom.

14. A compound having the formula



wherein Z^1 is NH, or NR_a, NR_a being C_{1-6} alkyl;

each of X^1 and X^2, independently, is O or S;

each of A^1 and A^2 is independently in the *(R)* or *(S)* configuration, and is independently H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

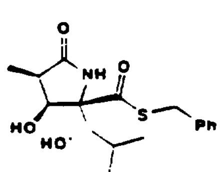
$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

15. A compound of claim 1, wherein Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 or $CHOR^1$, R^1 being H, halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl; R^2 is OR_f; and L^0 is $-(C=X^2)-L^1$, X^2 being O and L^1 being linked by a sulfur atom to the carbon atom bonded to X^2.

16. A compound of claim 15 wherein L^1 is selected from substituted or unsubstituted C_{6-20} arylalkylcarbonylthio, C_{6-20} arylthio, C_{2-8} alkylcarbonylthio, and C_{1-12} alkylthio.

17. A compound of claim 15 having a formula selected from:



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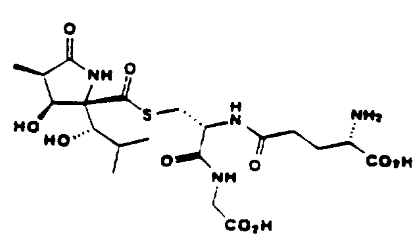
and



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18. A compound of claim 6 wherein Z^1 is O, S, SO_2, NH, or NR_a, R_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen, provided that when Z^1 is SO_2, X^1 is two singly bonded H;

Z^2 is CHR^1 in the *(R)* or *(S)* configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl);

Z^3 is O, S, NH, or NR_j, R_j being C_{1-6} alkyl;

X^2 is O or S; and

A^1 is the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C{=}NOR_h$, $-C{=}NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxy-

carbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; where CHX^4 is in the *(S)* configuration when X^4 is hydroxyl and $-(CH_2)_n-R^3X^3$ is isopropyl, and the moiety $-(CH_2)_n-R^3X^3$ has between 5 and 20 carbon atoms when X^4 is hydroxyl, m is 0, and Z^1 is NH.

19. A compound of claim 6 wherein Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 in the *(R)* or *(S)* configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl); Z^3 is O or NH; X^2 is O or S; and A^1 is the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-Y-(CH_2)_n-R^3X^3$$

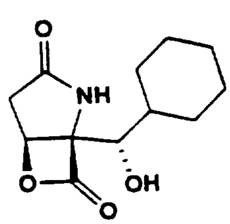
wherein Y is C=O or CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, or OR_h, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, or C_{6-10} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

20. A compound of claim 19, wherein Z^1 is NH and Z^3 is O.

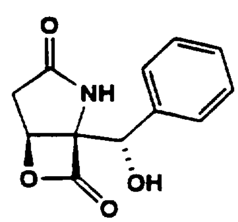
21. A compound of claim 19, wherein each of Z^1 and Z^3 is NH.

22. A compound of claim 6 having a formula wherein at least four of the following limitations apply: Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 in the (R) or (S) configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, hydroxyl, or OR_d, R_d being C_{1-6} alkyl; Z^3 is O or NH; X^2 is O; Z^3 is O or NH; and A^1 is the side chain of any naturally occurring α-amino acid, or CHX^4, X^4 being halogen, methyl, halomethyl, or OR_h, R_h being selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; m is 0, 1, or 2 and n is 0 or 1; and X^3 is H.

23. A compound of claim 22 selected from the following formulae:



J-1



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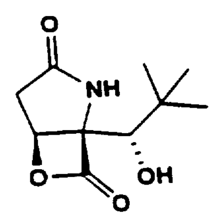

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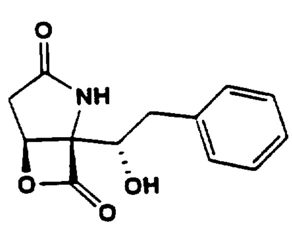

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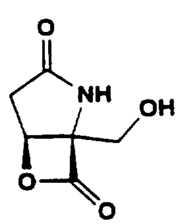

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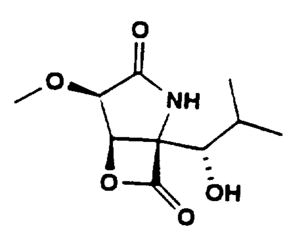

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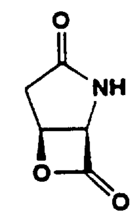

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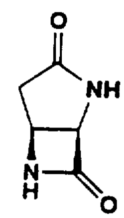

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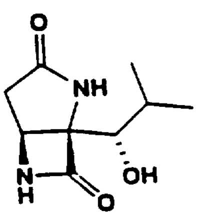

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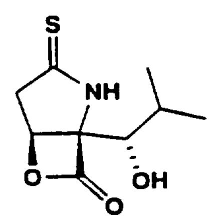

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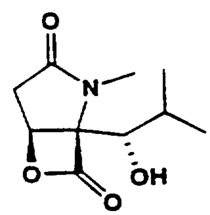

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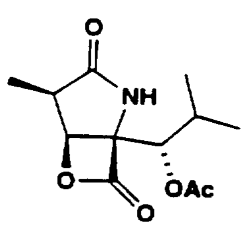

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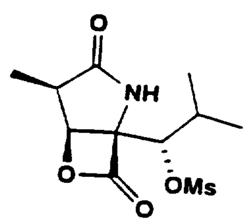

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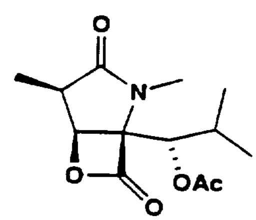

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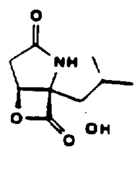

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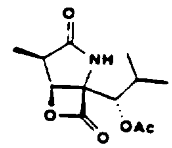

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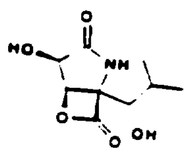

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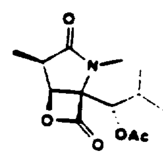

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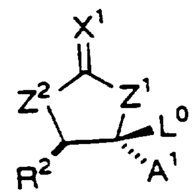
24. A pharmaceutical composition comprising a compound having the formula



wherein Z^1 is O, S, SO_2, NH, or NR_a, R_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen, X^1 being two singly bonded H when Z^1 is SO_2;

Z^2 is O, S, NH, NR_d, CHR^1, or $CHOR^1$ in the *(R)* or *(S)* configuration, wherein R_d is C_{1-6} alkyl and R^1 is H, halogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, NR_dR_e (except where Z^2 is $CHOR^1$), or the side chain of any naturally occurring α-amino acid, or R^1 and R^2 taken together are a bivalent moiety, provided that when R^1 and R^2 are taken together, Z^1 is NH or NR_a and Z^2 is CHR^1; R_e being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, and the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl, where the H in CHR^1 is deleted when R_1 and R_2 taken together form a C_{3-8} heteroaryl or C_{6-12} aryl;

R^2 is C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, azido, C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g(OR_f)$, or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H,

C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1 and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl, where the H in CHR^1 is deleted when R_1 and R_2 taken together form a C_{3-8} heteroaryl or C_{6-12} aryl;

A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

L^o is H or an organic moiety having 1 to 25 carbon atoms, 0 to 10 heteroatoms, and 0 to 6 halogen atoms; and

a pharmaceutically acceptable carrier.

25. A pharmaceutical composition of claim 24, wherein Z^2 is CHR^1 and Z^1 is NH or NR_a.

26. A pharmaceutical composition of claim 24,

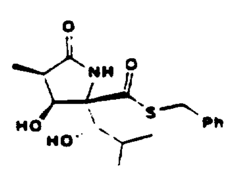
wherein L^0 is H or $-(C{=}X^2)-L^1$, X^2 being O and L^1 being linked by an oxygen or sulfur atom to the carbon atom bonded to X^2; where only one of A^1 and L^0 is selected from carboxyl and (C_{1-4} alkyl)oxycarbonyl; wherein L^1 is hydroxy, C_{1-12} alkoxy, C_{1-12} alkylsulfonyloxy, C_{6-20} arylsulfonyloxy, C_{7-20} arylalkyl, C_{6-20} aryloxy, C_{6-20} arylalkylcarbonyloxy, C_{2-8} alkylcarbonyloxy, C_{6-20} arylalkylcarbonylthio, C_{6-20} arylthio, C_{2-8} alkylcarbonylthio, or C_{1-12} alkylthio.

27. A pharmaceutical composition of claim 24, wherein L^1 is linked by a sulfur atom to the carbon atom bonded to X^2.

28. A pharmaceutical composition of claim 27, wherein L^1 is substituted or unsubstituted C_{6-20} arylalkylcarbonylthio, C_{6-20} arylthio, C_{2-8} alkylcarbonylthio, or C_{1-12} alkylthio.

29. A pharmaceutical composition of claim 24, wherein Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 or $CHOR^1$, R^1 being H, halogen, C_{1-6} alkyl, or C_{1-6} haloalkyl; R^2 is OR_f; and L^0 is $-(C{=}X^2)-L^1$, X^2 being O and L^1 being linked by a sulfur atom to the carbon atom bonded to X^2.

30. A pharmaceutical composition of claim 29, comprising a compound having a formula selected from:



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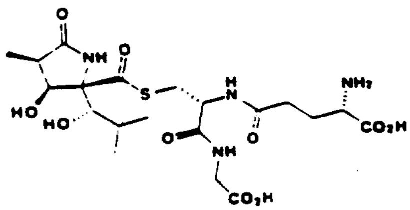
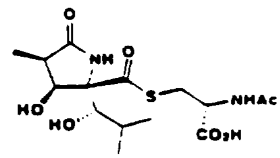


I-2

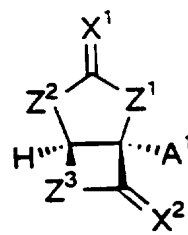
and



I-3 .

31. A pharmaceutical composition of claim 24, wherein Z^2 is O, S, NH or NR_d.

32. A pharmaceutical composition comprising a compound having the following formula



wherein Z^1 is O, S, SO_2, NH, or NR_a, R_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen, provided that when Z^1 is SO_2, X^1 is two singly bonded H;

Z^2 is CHR^1 in the *(R)* or *(S)* configuration, R^1 being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl);

Z^3 is O, S, NH, or NR_j, wherein R_j is C_{1-6} alkyl;

X^2 is O or S; and

A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

a pharmaceutically acceptable carrier.

33. A pharmaceutical composition of claim 32 wherein Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 in the *(R)* or *(S)* configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl); Z^3 is O or NH; X^2 is O or S; and A^1 is the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-Y-(CH_2)_n-R^3X^3$$

wherein Y is C=O or CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, or OR_h,

wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, or C_{6-10} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid.

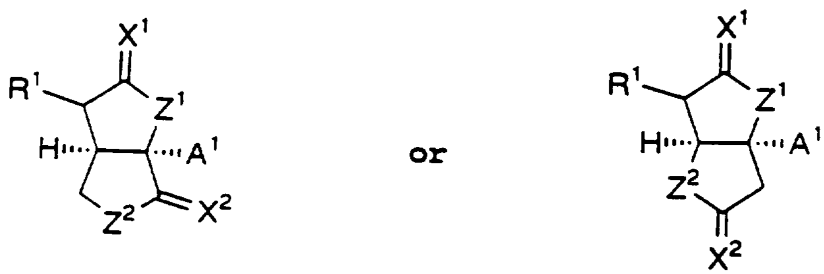
34. A pharmaceutical composition of claim 33, wherein X^2 is O, and A^1 is the side chain of any naturally occurring α-amino acid, or X^4 is halogen, methyl, halomethyl, or OR_h, R_h being selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; m is 0, 1, or 2 and n is 0 or 1; and X^3 is H.

35. A pharmaceutical composition of claim 32, comprising a compound having a formula selected from: clasto-lactacystin (9R)-β-lactone, clasto-lactacystin trifluoroethyl ester, descarboxylactacystin, lactacystin amide, and phenylacetyl lactacystin.

36. A pharmaceutical composition of claim 32, wherein Z^1 is NH or NR_a; X^1 is O or S; Z^2 is CHR^1 in the (R) or (S) configuration, wherein R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, hydroxyl, or OR_d, R_d being C_{1-6} alkyl; Z^3 is O or NH; X^2 is O; Z^3 is O or NH; and A^1 is the side chain of any naturally occurring α-amino acid, or CHX^4, X^4 being halogen, methyl, halomethyl, or OR_h, R_h being selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-10} acyl, C_{1-6} alkylsulfonyl, and C_{6-10} arylsulfonyl; R^3 is straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylene, or deleted; m is 0, 1, or 2 and n is 0 or 1; and X^3 is H.

37. A pharmaceutical composition of claim 32, comprising a compound having a formula selected from claim 23.

38. A pharmaceutical composition comprising a compound having a formula selected from:



wherein Z^1 is NH or NR_a, R_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen;

Z^2 is O, S, NH, or NR_j, wherein R_j is C_{1-6} alkyl;

R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occurring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen);

X^2 is O or S; and

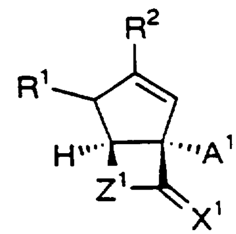
A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

a pharmaceutically acceptable carrier.

39. A pharmaceutical composition comprising a compound having the following formula



wherein Z^1 is O, S, NH or NR_j, R_j being C_{1-6} alkyl; X^1 is O or S;

R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-5} alkynyl);

R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-12} aryl, C_{3-8} heteroaryl, or halogen;

X^2 is O or S; and

A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

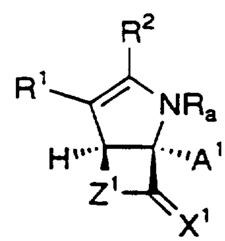
$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cyclo-

alkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

a pharmaceutically acceptable carrier.

40. A pharmaceutical composition comprising a compound having the following formula



wherein Z^1 is O, S, NH or NR_j, R_j being C_{1-6} alkyl;

X^1 is O or S;

R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of a naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen);

R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-12} aryl, C_{3-8} heteroaryl, or halogen;

R_a is C_{1-6} alkyl; and

A^1 is H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

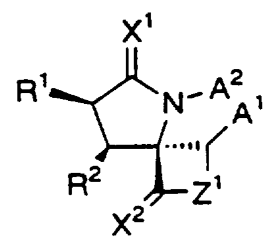
$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halo-

methyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxy-carbonyl, (C_{7-14} arylalkyl)oxy-carbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

a pharmaceutically acceptable carrier.

41. A pharmaceutical composition comprising a compound having the formula



wherein X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen;

Z^1 is O, S, NH, or NR_a, R_a being C_{1-6} alkyl;

R^1 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of any naturally occuring α-amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8}

heterocyclic radical, or halogen); or R^1 and R^2 taken together are a bivalent moiety which forms a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl;

R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, azido, C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g$-(OR_f), or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H, C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1 and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl;

X^2 is O or S; and

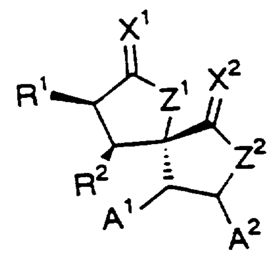
A^1 is in the *(R)* or *(S)* configuration, and each of A^1 and A^2 is independently H, the side chain of any naturally occurring amino α-acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, $(C_{1-6}$ alkyl)oxycarbonyl, $(C_{7-14}$ arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

a pharmaceutically acceptable carrier.

42. A pharmaceutical composition comprising a compound having the following formula



wherein Z^1 is NH or NR_a, NR_a being C_{1-6} alkyl;

X^1 is O, S, CH_2, two singly bonded H, $CH(R_b)$ in the E or Z configuration, or $C(R_b)(R_c)$ in the E or Z configuration, each of R_b and R_c, independently, being C_{1-6} alkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen;

Z^2 is O, S, NH, or NR_j, R_j being C_{1-6} alkyl;

R^1 is in the *(R)* or *(S)* configuration, and is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, hydroxyl, halogen, a side chain of any naturally occuring amino acid, OR_d, SR_d, or NR_dR_e (each of R_d and R_e, independently, being H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{6-12} aryl, C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or halogen); or R^1 and R^2 taken together are a bivalent moiety which forms a C_{3-8} cycloalkyl, C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl;

R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, azido, C_{2-6} alkynyl, halogen, OR_f, SR_f, NR_fR_g, $-ONR_fR_g$, $-NR_g-(OR_f)$, or $-NR_g(SR_f)$ (each of R_f and R_g, independently, being H, C_{1-6}, alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl), or R^1 and R^2 taken together are a bivalent moiety, the bivalent moiety forming a C_{3-8} cycloalkyl,C_{3-8} heteroaryl, C_{3-8} heterocyclic radical, or C_{6-12} aryl;

X^2 is O or S; and

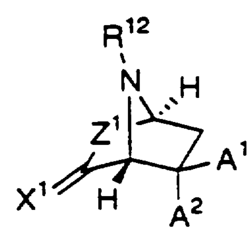
each of A^1 and A^2 is independently in the *(R)* or *(S)* configuration, and is independently H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

a pharmaceutically acceptable carrier.

43. A pharmaceutical composition comprising a compound of the formula



wherein Z^1 is O, NH, or NR_a, NR_a being C_{1-6} alkyl; X^1 is O, S, CH_2, or two singly bonded H;

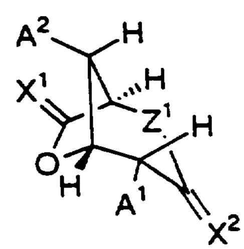
each of A^1 and A^2 is independently H, the side chain of any naturally occurring α-amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and

R^{12} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl; and a pharmaceutically acceptable carrier.

44. A pharmaceutical composition comprising a compound having the formula



wherein Z^1 is NH, or NR_a, NR_a being C_{1-6} alkyl; each of X^1 and X^2, independently, is O or S;

each of A^1 and A^2 is independently in the *(R)* or *(S)* configuration, and is independently H, the side chain of any naturally occurring amino acid, or is of the following formula,

$$-(CH_2)_m-Y-(CH_2)_n-R^3X^3$$

wherein Y is O, S, C=O, C=S, -(CH=CH)-, vinylidene, $-C=NOR_h$, $-C=NNR_iR_{i'}$, sulfonyl, methylene, CHX^4 in the *(R)* or *(S)* configuration, or deleted, X^4 being halogen, methyl, halomethyl, OR_h, SR_h, $NR_iR_{i'}$, $-NR_i(OR_h)$, or $-NR_i(NR_iR_{i'})$, wherein R_h is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, and each of R_i and $R_{i'}$, independently is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-10} acyl; m is 0, 1, 2, or 3, and n is 0, 1, 2, or 3; and R^3 is straight chain or branched C_{1-8} alkylidene, straight chain or branched C_{1-8} alkylene, C_{3-10} cycloalkylidene, C_{3-10} cycloalkylene, phenylene, C_{6-14} arylalkylidene, C_{6-14} arylalkylene, or deleted, and X^3 is H, hydroxyl, thiol, carboxyl, amino, halogen, (C_{1-6} alkyl)oxycarbonyl, (C_{7-14} arylalkyl)oxycarbonyl, or C_{6-14} aryl; or R^3 and X^3 taken together are the side chain of any naturally occurring α-amino acid; and a pharmaceutically acceptable carrier.

45. A method of treating inflammation, comprising administering to a subject an effective anti-inflammatory amount of a pharmaceutical composition of claim 24 or claim 32.

46. A method of claim 45, wherein the inflammation is associated with injury or infection.

47. A method of claim 45, wherein the inflammation is associated with an allergy or asthma.

48. A method of claim 45, wherein the inflammation is associated with a disease state selected from rheumatoid arthritis, scleroderma, rheumatic fever, inflammatory bowel disease, diabetes mellitus, myasthenia gravis, multiple sclerosis, Guillan-Barre syndrome, conjunctiva of the eye, systemic lupus erythematosus, encephalitis, Adult Respiratory Distress Syndrome, emphysema, Alzheimer's disease, and muscular dystrophy.

49. A method of claim 45, wherein the inflammation is associated with transplantation of bone marrow or a solid organ selected from kidney, lung, heart, pancreas, liver, and skin, and the composition is administered before, during, or after transplantation.

50. A method of claim 45, wherein the pharmaceutical composition is administered orally.

51. A method of claim 45, wherein said composition is a composition of claim 34.

52. A method of claim 45, wherein said composition is a composition of claim 30.

53. A method of treating cancer, comprising administering to a subject an effective anti-cancer amount of a pharmaceutical composition of claim 24 or 32.

54. A method of claim 53, wherein the cancer is selected from carcinoma, lymphoma, sarcoma, and myeloma.

55. A method of claim 53, wherein said cancer is selected from adenocarcinoma, acinic cell adenocarcinoma, adrenal cortical carcinomas, alveoli cell carcinoma, anaplastic carcinoma, basaloid carcinoma, basal cell car-

cinoma, bronchiolar carcinoma, bronchogenic carcinoma, renaladinol carcinoma, embryonal carcinoma, anometroid carcinoma, fibrolamolar liver cell carcinoma, follicular carcinomas, giant cell carcinomas, hepatocellular carcinoma, intraepidermal carcinoma, intraepithelial carcinoma, leptomanigio carcinoma, medullary carcinoma, melanotic carcinoma, menigual carcinoma, mesometonephric carcinoma, oat cell carcinoma, squamal cell carcinoma, sweat gland carcinoma, transitional cell carcinoma, tubular cell carcinoma, amelioblastic sarcoma, angiolithic sarcoma, botryoid sarcoma, endometrial stroma sarcoma, ewing sarcoma, fascicular sarcoma, giant cell sarcoma, granulositic sarcoma, immunoblastic sarcoma, juxaccordial osteogenic sarcoma, Kaposi's sarcoma, leukocytic sarcoma, lymphatic sarcoma, medullary sarcoma, myeloid sarcoma, austiogenci sarcoma, periosteal sarcoma, reticulum cell sarcoma, round cell sarcoma, spindle cell sarcoma, synovial sarcoma, and telangiectatic audiogenic sarcoma, neural blastoma, glioblastoma, astrocytoma, melanoma, leiomyo sarcoma, multiple myeloma, Hemangioma, Hodgkin's disease, Burkitt's lymphoma, and nodular poorly-differentiated lymphocytic lymphoma, nodular mixed lymphocytic lymphoma, nodular histiocytic lymphoma, and diffuse lymphomas.

56. A method of claim 53, wherein said composition is a composition of claim 34.

57. A method of claim 53, wherein said composition is a composition of claim 37.

58. A method for treating psoriasis, comprising administering to a subject an effective amount of a pharmaceutical composition of claim 24 or 32.

59. A method for treating restenosis, comprising administering to a subject an effective amount of a pharmaceutical composition of claim 24 or 32.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05072

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/395;38/00
US CL :514/210,13,14,15
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/210,13,14,15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN COMPOUNDS AND VARIOUS METHODS OF USE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,453,502 (AIKINS ET AL) 26 September 1995. see entire documrnt.	1-44, 46, 48
Y	HETEROCYCLES, Volume 20, number 12, issued 1983, Kametani et al, "STUDIES ON THE SYNTHESIS OF CARBAPENEM ANTIBIOTICA. STEREOSLECTIVE SYNTHESIS OF POTENTIAL INTERMEDIATE FOR 6-AMIDOCARBAPENEM ANTIBIOTICS"pages 2355-58, see entire document	1-44,46,48

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 24 JULY 1996	Date of mailing of the international search report 26 AUG 1996
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